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THE GENUS HELICOCERAS

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The members of the genus *Helicoceras* were formerly placed in the genus *Gyroceras*. A study of the literature, however, shows that this latter name cannot be applied to the species other than *Gyroceras ammonis*, and for reasons to be shown shortly that name is not valid because of synonymy.

The genus *Gyroceras*, based on *G. ammonis*, was erected by Corda[1] in 1837. His description of the original species runs as follows: "Acervulis atris, confluentibus, stromatis strato inferiore atro; superiore luteo, hyalino, celluloso; floccis infra atris, supra attenuatis luteis; sporis ovalibus discoideis, depressis." It readily can be seen that the presence of a two-layered stroma sharply sets off Corda's species from the non-stromatic ones that make up the remainder of the genus. These latter are parasitic or weakly so, and their vegetative hyphae, although branching, never aggregate to form a stroma under the sporogenous area. A careful examination of the original figure illustrating *G. ammonis* makes it obvious that the present-day conception of the genus is founded on misinterpretation. The "strato superiore" is definitely a layer of short conidiophores beyond which the curved sterile hyphae project. According to Corda, these sterile hyphae break into ovoid discoid spores, a statement that seems partly to have been the cause of the misconception of his genus, and one that needs confirmation before final acceptance. Even though this doubtful point were settled, Corda's genus is not that of later investigators and, in fact, should be relegated to synonymy, as is done in the

[1] Corda, A. C. I., Icon. Fung. 1: 9. *pl. 2, fig. 141.* 1837.

present paper, and the species placed in the genus *Sarcopodium* of Ehrenburg[1] under the subgenus *Tricholeconium* as it was understood by Lindau.[2]

In 1856, when Montagne and Cesati[3] transferred *Monilia Celtidis* (*Celtis*) to *Gyrocerus*, they established the present conception of the genus. Although they did not give a generic description, they did describe the species rather fully. In the description it is stated that "stroma adest tenuissimum, a Bivona praetermissum, e fibrillis exilissimis materiae grumulosae intermixtus constans, pallidum," and further that the spore filaments "tandem in sporas globosas secedentis." The term stroma is obviously misapplied in this instance, so much so that it would appear that the authors were stretching the term so as to make the species fit into the genus. The statement that the spore filament breaks into globose spores can be substantiated neither by the investigations of *Gyroceras Celtidis* by Killian,[4] nor by the writer's observations on *Helicoceras Oryzae*.

Saccardo[5] in 1886 accepted Montagne and Cesati's interpretation of Corda's genus, and in his generic description perpetuated the erroneous statement that the conidial filament breaks into spores. At the same time he changed the generic name from *Gyrocerus* to *Gyroceras* for etymological reasons and accredited the genus to Corda.

Despite the fact that Saccardo's interpretation of Corda's genus was accepted by Massee,[6] Lindau,[7] and others, according to the rules of nomenclature, when the type species becomes a synonym, the name of the genus also falls into synonymy. The writer therefore proposes the new name *Helicoceras*.

Helicoceras Linder, n. nom.

Gyroceras Corda, Icon. Fung. **1**: 9. *pl. 2, fig. 141.* 1837, of authors, in part.

[1] Ehrenburg, C. G., Silv. Myc. Berol. pp. 12, 23. *fig. 24.* 1818.

[2] Lindau, G. in Rabenhorst, L., Kryptog. Fl. 2nd. ed. 1(8): 708. 1906.

[3] Montagne and Cesati in Montagne, J. F. C., Syll. Gen. Spec. Cryptogam. p. 308. 1856.

[4] Killian, Ch., Soc. d'Hist. Nat. Afrique Nord, Bull. **60**: 274–281. 1925.

[5] Saccardo, P. A., Syll. Fung. **4**: 266. 1886.

[6] Massee, G., Brit. Fungus Fl. **3**: 365. *fig. 11*, p. 313. 1893.

[7] Lindau, G., in Engler & Prantl, Nat. Pflanzenfam. **1****: 459. *fig. 273a.* 1900; and in Rabenhorst, L., Kryptog. Fl. 2nd ed. 1(8): 605. 1906.

Mycelia sterilia in substrato extensa, ramosa, septata, non in stromatem aggregata; conidiophoris ex mycelio repente, rectis vel curvis, simplicibus vel ad apices breve-ramosis, apicibus inflatis vel non inflatis; conidiis atris, fuscis, vel subfuscis, multiseptatis, ad septa constrictis, irregulariter curvis, recurvis, vel glomeratis, levibus vel echinulatis.

Sterile mycelium extensive in the substratum, not forming a stromatic layer, branched, septate; conidiophores arising as lateral, erect or ascending branches from the creeping mycelium, simple or terminally short-branched, inflated apically or nearly isodiametric; conidia dilute to deep fuscous, multiseptate, constricted at the septa, irregularly bent, strongly recurved, to two times helically coiled, echinulate or smooth.

The type species is *Helicoceras Celtidis* (Biv.-Bernh.) Linder.

As at present constituted, *Helicoceras* contains four species. Of these, three are parasitic or weakly so. Their economic importance is not great since the hosts attacked are of minor value, with the limited exception of cultivated water-lilies which are occasionally severely damaged by *Helicoceras Nymphaearum*. The four members fall into two equal groups, one characterized by relatively smooth, the other by echinulate, conidia. The resemblance between the two groups is so great, however, that the creation of an additional genus for the two echinulate-spored species would add nothing to the ease of classification of so small a group.

KEY TO THE SPECIES OF HELICOCERAS

1. Conidia smooth, cells shorter than wide; conidiophores not conspicuously inflated nor densely branched at the apices.....2
1. Conidia echinulate, cells longer than wide; conidiophores mostly inflated at the apex and often densely short-branched.....3
2. Conidia 5–7.5–(9) μ thick. On *Celtis* spp.....*1. H. Celtidis*
2. Conidia 8–13 μ thick. On *Plantago* spp.....*2. H. Plantaginis*
3. Conidia 60–190 × 5–18 μ. On *Nymphaea* spp.....*3. H. Nymphaearum*
3. Conidia 64–90 × 5.4–9 μ. On seeds of *Oryza*.....*4. H. Oryzae*

1. Helicoceras Celtidis (Biv.-Bernh.) Linder, n. comb.

Monilia Celtidis (*Celtis*) Bivona-Bernhardi, Stirp. Rar. in Sicilia sponte proven. **3**: 18. *pl. 3, fig. 6.* 1813.

Gyroceras Celtidis (Biv.-Bernh.) Mont. & Ces., in Montagne, J. F. C., Syll. Gen. Spec. Cryptogam. p. 308. 1856.

Gyroceras divergens Peck, Torr. Bot. Club Bull. **36**: 155. 1909.

Plate 1, figs. 9–16.

Mycelium light fuscous to fuscous, branched, septate, penetrating through the host tissues. Conidiophores as short branches of the mycelium, simple, little differentiated. Conidia fuscous, curved, circinate, or once-coiled, more conspicuously coiled in dried material, multiseptate, some cells occasionally diagonally or longitudinally septate, constricted at the transverse septa, 50–100 × 5–8 μ, the cells shorter than wide.

Parasitic on leaves of *Celtis* spp., also reported[1] on leaves of *Sponias sinensis*. Europe, North America, and Japan.

Occasionally the outer walls of the spore cells are ruptured and this may give to the spore a false appearance of echinulation. The color of the colonies is fairly constant in all specimens examined. There is, however, in the Sydow Herbarium at Stockholm a form of this species of which the spores are brick-red in color and which is labelled *G. Celtidis* forma *fulvescens*. Excepting for the color of the spores, this material agrees in all details with the typical specimens. *Gyroceras divergens* of Peck is in no way different from the European material.

Specimens examined:

Exsiccati: D. Saccardo, Myc. Ital., 1581; P. A. Saccardo, Myc. Veneta, 276; Kabat & Bubak, Fungi Imp. Exsicc., 395; H. Sydow, Myc. German., 1294; Rabenhorst, Herb. Myc., 275; E. Bartholomew, Fungi Columb., 3525; Seymour & Earle, Econ. Fungi, 147.

United States:

Arkansas: Batesville, *Bartholomew*, in Fungi Columb.

Missouri: Elmwood, *Demetris*, in Kabat & Bubak, Fungi Imp. Exsicc.

Kansas: Manhattan, *Galloway, 1176*, in Seymour & Earle, Econ. Fungi.

Italy: Pedemont, *Cesati*, in Rabenhorst, Herb. Myc. (probably authentic material); Treviso, *P. A. Saccardo*, in Myc. Venet.

Japan: Tokyo, *Shirai*, as *G. Celtidis* forma *fulvescens* (Stockholm).

[1] Lindau, G., in Rabenhorst, L., Kryptog. Fl., 2nd ed. 1(8): 606. 1906.

2. Helicoceras Plantaginis (Cda.) Linder, n. comb.

Torula plantaginis Corda, Icon. Fung. **3**: 5. *fig. 14*. 1839.

Gyroceras Plantaginis (Cda.) Saccardo, Michelia **1**: 266. 1878.

Plate 1, figs. 17–20.

Mycelium light to deep fuscous, branched, septate, 3–4.5 μ diam. Conidiophores fuscous, as side branches of the vegetative mycelium, occasionally branching terminally (pl. 1, fig. 20), little differentiated. Conidia deep fuscous to almost black, bent or slightly coiled, more pronouncedly coiled in dry material, smooth, simple or branched (pl. 1, fig. 19), multiseptate, constricted at the septa, 50–110 × 7–10 μ, the cells shorter than wide.

On old living leaves of *Plantago* spp. Widespread in Europe.

This species appears to be a weak parasite that only attacks the senescent leaves of the various species of *Plantago*. It is definitely delimited by the host it infects, and the color and size of the conidia.

Specimens examined:

Exsiccati: Wartmann & Schenk, Schweiz. Kryptog., 617; H. Sydow, Myc. German., 1294; Fuckel, Fungi Rhenan., 65.

France: Lorraine near Forbach, *Ludwig*, in Sydow, Myc. German.

Germany: Munchau, in Fuckel, Fungi Rhenan.

Switzerland: Bern, in Wartmann & Schenk, Schweiz. Kryptog.

3. Helicoceras Nymphaearum (Rand) Linder, n. comb.

Helicosporium Nymphaearum Rand, Jour. Agr. Res. **8**: 219–232. *pl. 67–70*. 1917.

Gyroceras Nymphaearum (Rand) Linder, Mo. Bot. Gard. Ann. **16**: 294–295. 1929.

Plate 1, figs. 5–8.

Mycelium intercellular, light brown, often hyaline in culture, septate, and branched. Conidiophores slender, 2–3 μ in diameter, of varying length, inflated at the apices, 6–7.5 μ, often becoming much short-branched apically and thus producing conidia in clusters. Conidia 60–170–(190) × (5)–6.3–14.4–(18) μ, brown, multiseptate, strongly constricted at the septa, the apical cells often subspherical or ovoid, the basal cell rounded-tapering, the

remaining cells longer than wide, minutely echinulate to finely tuberculate.

Parasitic on leaves of *Nymphaea* spp. New York, New Jersey, and Washington, D. C.

In a previous paper (*l. c.*), the writer, through an error, stated that the sclerotia reported by Rand are rounded, subcarbonaceous, and measure 150–190 μ in diameter. The measurements should read 150–900 μ in diameter.

Specimen examined:

United States:

Washington, D. C.: *Rand*, TYPE (U. S. Dept. Agr. and slide in Farlow Herb.).

4. Helicoceras Oryzae Linder & Tullis, n. sp.

Plate 1, figs. 1–4.

Mycelium hyalinum vel albido-fuscum, septatum, ramosum, 1.5–5.4 μ diam.; conidiophoris subhyalinis vel hyalinis, laevibus, simplicibus vel ad apices inflatos breve-ramosis, 1.8–5.4 μ diam., ad extremos 5.4–7.4 μ diam.; conidiis echinulatis, subfuscis, multiseptatis, in septis constrictis, curvatis vel subhelicoideis, in basi et apice rotundatis, 64–90 × 5.4–9 μ.

Vegetative mycelium creeping, hyaline to light fuscous, septate, branched, 1.5–5.4 μ in diameter. Conidiophores subhyaline to hyaline, smooth, simple or short-branched at the inflated apices, of varying length, 1.8–5.4 μ thick, enlarging terminally to 5.4–7.4 μ. Conidia curved or somewhat helically coiled, light fuscous, multiseptate, constricted at the septa, the cells longer than wide, mostly of equal diameter, echinulate, the basal cell abruptly rounded, 64–90 × 5.4–9 μ

On kernels of Chinese rice. Texas.

This species was communicated to the writer by Professor E. C. Tullis, of the University of Arkansas, who isolated it from a kernel of Chinese rice sent to him from Texas. There is no information concerning the pathogenicity of this species.

Superficially, *H. Oryzae* resembles *H. Nymphaearum* and, like that species, also produces small sclerotia on certain media. The conidia when viewed under a hand lens appear either fulvous or fuscous, depending upon their age. The spores of this species are

smaller than are those of the related one, not so deeply constricted at the septa, and the cells are more uniform in size.

Specimen examined:

United States:

Texas: *E. C. Tullis.* **TYPE** (slides in Mo. Bot. Gard. Herb., the Farlow Herbarium, and the writer's herbarium).

Excluded Species

Gyroceras ammonis Corda, Icon. Fung. **1**: 9. *pl. 2, fig. 141.* 1837. = **Sarcopodium** (Tricholeconium) **ammonis** (Cda.) Linder, n. comb.

Gyroceras saxonicum Lindau, in Rabenhorst, L., Kryptog. Fl., 2nd ed. **1**(8): 606. 1906. = *Coremiella saxonicum* (Lindau) Feurich, Isis Budissina Bautzen **11**: 137. 1928.

Explanation of Plate

PLATE 1

The drawings are made with the aid of a camera lucida. The magnifications in all cases are × 500.

Figs. 1–4. *Helicoceras Oryzae* Linder & Tullis.

In figs. 1 and 2 are shown the typical spores of the species and also variations in the conidiophores. Fig. 4 illustrates a much-branched conidiophore that bears three immature two-celled spores.

Figs. 5–8. *Helicoceras Nymphaearum* (Rand) Linder.

In these figures may be seen the various types of conidiophores, from the simple to the much-branched. The unequal sizes of the cells of the conidia are clearly brought out, as are also the deep constrictions at the septa.

Figs. 9–16. *Helicoceras Celtidis* (Biv.-Bernh.) Linder.

The fulvous form of the species is shown in figs. 9–12. The branched conidiophores shown by fig. 9 are also found in the typical material. In fig. 16, the exospore has ruptured, exposing the lighter-colored endospore.

Figs. 17–20. *Helicoceras Plantaginis* (Corda) Linder.

The simple type of conidiophore may be seen in fig. 17, and a more loosely branched conidiophore in fig. 20. A branched conidium is also depicted in fig. 20. Such branching of conidia is of relatively rare occurrence.

LINDER — HELICOCERAS

BRIEF NOTES ON THE HELICOSPOREAE WITH DESCRIPTIONS OF FOUR NEW SPECIES

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Since the publication of "A monograph of the helicosporous Fungi Imperfecti,"[1] the writer has had an opportunity to study additional material of this interesting and beautiful group of Fungi Imperfecti. The material was communicated by Professor G. W. Martin of the University of Iowa and by Mr. John Dearness of Toronto, Canada, and to them the writer wishes to express his sincere thanks. In addition to the material mentioned above, the writer, while examining his collection of Fungi Imperfecti, discovered four additional species: two from the tropics, one from Missouri, and one from Alabama. These are to be described later in this paper.

In addition to the description of the four species, these notes are intended primarily to amplify observations on species which have hitherto been collected only once or twice, and secondarily for the purpose of adding localities and thus enlarging the range of the species as given in the previous paper.[1]

Helicosporium griseum (Bon.) Sacc. This species is reported from North America for the first time and is represented by two collections. The material from Iowa (*T. A. MacBride*, Iowa City, 1909 (Ia.)[2]) agrees very well with the European material. The spore filaments, however, are somewhat thicker (1.4 μ). The Canadian specimen (*Thos. Langton*, Toronto (Ia.)) is almost identical with the Iowan material. It differs only in producing conidia more abundantly and higher on the conidiophores, the latter a character more in agreement with the species as it is delineated by Bonorden[3] in the original figure. The variations between the two collections are very slight and could probably be explained by differences in the environmental conditions.

Helicosporium phragmites v. Höhnel. Although no data were given on the label of this specimen, it appears probable that it was

[1] Linder, D. H. Mo. Bot. Gard. Ann. **16**: 227–388. *pl. 12–31, 17 text figs.* 1929.

[2] The specimens from Professor Martin, deposited in the herbarium of the University of Iowa, are indicated by (Ia) and those from Mr. Dearness by (D).

[3] Bonorden, H. F. Handbuch, p. 74. *fig.* 77. 1851.

collected by MacBride in Iowa (Ia). As is true with the other American collection from Kittery Point, Maine, no perithecial stage was found with the imperfect stage, although it is in close agreement with the European material. The material at hand, as well as that from Kittery Point, strongly suggests an attenuated form of *H. lumbricoides*, but the colonies are not so dense nor are they so readily separable from the substratum. It is interesting to note that this species appears to favor a particular type of substratum,—the Iowan material occurring on old corn stalks, that from Kittery Point on *Carex* stalks, while that from Austria on *Phragmites*.

Helicomyces bellus Morgan. The status of this species becomes very doubtful when material, identified by Morgan, is studied. For example, one specimen (*Morgan*, Ohio, 1902 (Ia)) is clearly *Helicomyces roseus* Lk., while the other (*Morgan*, Ohio?, 1909 (Ia)) is *Helicosporium lumbricopsis* Linder. This latter material can in no manner be considered to belong under *Helicomyces bellus* since Morgan[1] emphasizes the repent character of the hyphae as follows: "The hyphae creep close to the substratum and are nearly concealed by the abundant spores . . . ," and also, "Hyphae creeping, septate, branched, brownish-hyaline, bearing spores on minute lateral teeth." In the material here discussed, the conidiophores are ascending and anastomose after the fashion of *H. lumbricopsis*. Since there is no type material, or at least since it is not available at present, the identity of Morgan's species must remain in doubt.[2]

Helicoma ambiens Morgan. When this species was studied during the preparation of the writer's monograph, only a single specimen was available. Recently two additional collections have been studied, one from Iowa (North Liberty, 1905 (Ia)) and another from Canada (*Dearness*, London, Ontario, 1893 (Ia)), and in both the branching character of the conidiophores and the bluntly rounded, recurved basal end of the conidia proved to be very satisfactory diagnostic characters for the separation of the species from *Helicoma Curtisii*. This latter species is also represented by a collection from Iowa (*T. H. MacBride*, 1889

[1] Morgan, A. P. Cinci. Soc. Nat. Hist. Jour. 15: 42. *fig. 4.* 1892.
[2] See Note at end of this paper.

(Ia)). The material is at a very advanced stage of development in which the conidiophores have become loosely aggregated to form loose fascicles, within which there are occasional anastomoses between the elements. There is not, however, any evidence of the branching that is so typical of *H. ambiens*.

From Canada an additional station is reported for *Helicoma olivaceum* (Karst.) Linder (*G. K. Bisby*, Winnipeg, May 26 (D)), and for *Helicoon ellipticum* (Pk.) Morgan (*Johnson & Bisby*, Winnipeg, Oct. 25, 1927 (D)). The material is typical of the species represented.

Helicoma repens Morgan. This material, collected and named by Morgan, was recently made available for study for the first time. Since it does not agree entirely with the original description[1] it seems desirable to repeat it and to indicate changes by *italics*.

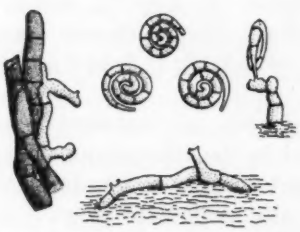


Fig. 1. *Helicoma repens*. × 500.

"Effused, forming a minutely flocculose, pinkish stratum. Hyphae creeping, *or scandent on hyphae of other fungi*, septate, hyaline, with very short ascending branches, which are covered by the abundant spores. Spores hyaline, multiguttulate, *10–16-times septate*, coiled nearly 2½ times; the coil 18–21 (*12–15*) mic. in diameter; the thread 80–100 mic. in length, about 4 (*2–3*) mic. thick; the inner extremity obtuse, the outer (*basal*) long and tapering."

This species closely resembles *Helicoma polysporum*, but is easily separated from it by the more frequently septate spores and the thinner spore filaments. The conidiophores of the two species are much alike. In *H. repens* the conidiophores not only are creeping but, as shown in the accompanying text-figure, are

[1] Morgan, A. P., *l. c.* p. 47. *fig. 12.*

also scandent on the hyphae of other fungi,—in this case on *Helicoma ambiens*.

The specimen studied (*Morgan*, Ohio?, 1887) has been designated as the type, and is deposited in the mycological herbarium of the University of Iowa at Iowa City.

The two following species from the American tropics, one from Missouri, and one from Alabama are described for the first time.

Helicoma Westoni Linder, sp. n.

Plate 2, figs. 1–3.

Mycelia sterilia in substratu immersa; conidiophoris fuscis vel ad apices albido-fuscis, simplicibus, rectis vel curvis, subinde geniculatis, 171–216–(252) μ longis, in basis 7.2–9 μ crassis, in apicibus 5.4–7.2 μ crassis; conidiis acrogenis, subinde pleurogenis, sessilibus, albido-fuscis, in 1½–1¾ spiras convolutis, 11–14-septatis, septis hyalinis; filis conidiorum laeviter fastigatis, basis truncatis, apicibus rotundatis, 11.5–13.5 μ crassis; spiris 33.5–38 μ diam.

Colonies inconspicuous, of scattered conidiophores. Sterile mycelium imbedded in the substratum. Conidiophores fuscous below, dilute or light fuscous at terminal cells, simple, erect or ascending, occasionally geniculate where first spores were produced, 171–216–(252) μ long, 7.2–9 μ thick at base, 5.4–7.2 μ above. Conidia acrogenous, less frequently tardily dehiscent and then pleurogenous, sessile, dilute fuscous, 1½–1¾-times tightly coiled, 11–14-times septate, septa hyaline, filament tapering slightly towards the ends, the basal end truncate, the distal end abruptly rounded, 11.5–13.5 μ thick, the coiled conidia 33.5–38 μ diam.

On decaying sheath of cocoanut palm. Trinidad, B. W. I.

This species is dedicated with great pleasure to Professor William H. Weston, Jr., of Harvard University, as a token of gratitude for the inspiring instruction and the kindly and generous assistance given to the writer while a student.

No species is comparable to this one, since all others with conidia similar to those of *H. Westoni* produce their spores on distinct sporogenous teeth. In this species, the conidia not only

are sessile, but are also provided with a distinct hyaline upward-tapering collar at the base of the spore filament.

Specimen examined:

Trinidad, B. W. I.: St. Augustine, *Linder, 15.* TYPE (in Farlow Herbarium of Harvard University).

Helicoma anastomosans Linder, sp. n.

Plate 2, figs. 4–9.

Coloniae effusae, flocculosae, dilute-roseae; conidiophoris albido-fuscis, pellucidis, simplicibus, rectis vel curvis, parce ramosis vel anastomosis, (20)–30–60–(100) × 3.6–5.5–(6.5) μ; conidiis acrogenis, raro pleurogenis, ad dentes gracilis conspicuos, hyalinis, 18–25-septatis, septis hyalinis, filis in 1½–1¾ spiras convolutis, 3.5–4 μ crassis; spiris 19.8–23.4 μ diam.

Colonies effuse, flocculose, pinkish. Conidiophores dilute fuscous, pellucid, simple, erect or ascending, sparsely branched, anastomosing, (20)–30–60–(100) × 3.6–5.5–(6.5) μ. Conidia acrogenous, less frequently pleurogenous, obliquely attached to conspicuous slender cylindrical sporogenous teeth, hyaline, 18–25-times septate, the septa hyaline; filament 1½–1¾ times coiled, 3.5–4 μ thick, the coiled conidia 19.8–23.4 μ diam.

On decaying manicole palm. British Guiana.

Although resembling *H. Morgani* in its spore characters, this species is quite distinct. The conidiophores, instead of being rather elongate and loosely branching, are short and simple, and anastomose frequently. The sporogenous teeth are prominent and cylindrical. Such characters, although seemingly of minor importance, are remarkably constant and separate this species quite clearly. Still another character is the method in which the conidia are produced. In *H. Morgani*, although two spores may be borne on or near the end of a conidiophore at the same time, they are not as a rule of equal age and hence when both conidia are mature, one, the older, is somewhat lower on the conidiophore than the other. In this species, two or occasionally more conidia are produced almost simultaneously, so that the spores appear frequently in pairs at an equal height on the conidiophore, generally at the apex.

Specimen examined:

British Guiana: Plantation Vryheid, *Linder, 836.* TYPE (in Farlow Herbarium of Harvard University).

Helicoma tenuifilum Linder, sp. n.

Plate 2, figs. 10–13.

Coloniae effusae, "Dark Olive" vel "Chaetura Drab";[1] conidiophoris fuscis vel albido-fuscis, ad cellulas extremas hyalinis, rectis vel curvis, ramosis vel multi-ramosis, perraro anastomosis, 25–60–(80) × 3.6–5 μ; conidiis acrogenis, raro pleurogenis, ad dentes gracilis fastigatos, hyalinis, 18–25-septatis, septis hyalinis, filis in 2¾–3¼ spiras convolutis, 2.5–3.6 μ crassis; spiris 21–28 μ diam.

Colonies effuse, velvety, with age becoming matted, "Dark Olive" to "Chaetura Drab." Conidiophores fuscous to light fuscous below, dilute fuscous to hyaline at the terminal cells, erect or ascending, branched to much branched, very rarely anastomosing, 25–60–(80) × 3.6–5 μ. Conidia acrogenous, less frequently pleurogenous, obliquely attached to short, tapering, sporogenous teeth, hyaline, 18–25-times septate, the septa hyaline; filament 2¾–3¼ times coiled, 2.5–3.6 μ thick, the coiled conidia 21–28 μ diam.

On decaying bark of *Carya?*. Missouri.

With *H. violaceum*, *H. Morgani*, and *H. anastomosans*, *H. tenuifilum* constitutes a rather homogeneous section in the genus *Helicoma*. The four species are characterized by the same type of conidia, and, with the exception of *H. violaceum*, the conidia are attached obliquely to the sporogenous teeth. *H. tenuifilum*, as the name implies, has more slender conidial filaments that are coiled more times. In addition, the conidia are produced singly. The conidiophores also are characteristic in that they are more richly branched, the branches never exceeding the length of the main axis of the conidiophores, as in *H. Morgani*. Occasionally the main axis of the conidiophore elongates (pl. 2, fig. 10) and later becomes much branched on the lower portions, thus resembling a fascicle of conidiophores. Fascicles, however, are not

[1] Ridgway, R. Color standards and nomenclature. Washington, D. C., 1912.

of rare occurrence in the older parts of the colony and give to it its matted appearance. "Sclerotes pedicelées" are present and in some instances suggest perithecia initials.

Specimen examined:

Missouri: Allenton, Oct. 1929, *Linder*, TYPE. (Mo. Bot. Gard. Herb., 68076, and in the writer's herbarium).

Helicomyces fuscopes Linder, sp. n.

Text-fig. 2.

Colonia effusa, stratum tenue, albidum formans; myceliis sterilibus fuscis in substrato immersis vel ad superficiem applicatis; conidiophoris dilute fuscis, rectis, simplicibus vel propter dentes sporigeros conspicuos ad apicem simulate breve ramosis, 1–3-septatis, 18–39–(50) × 2.5–3.6 μ; conidiis hyalinis, acrogenis vel

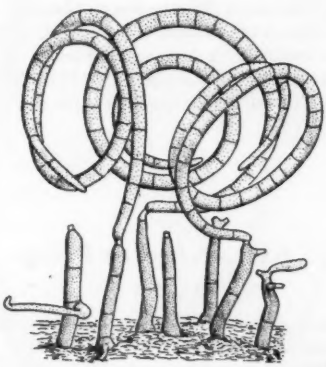


Fig. 2. *Helicomyces fuscopes.* × 500.

aliquando pleurogenis, multiseptatis; filis in spiras 1¼–2¼ convolutis, 3.6–4.5 μ crassis, ad extrema exteriora fastigatis, ad bases rotundatis et oblique complanatis; spiris 39.5–62 μ diam.

Colony effused, forming a thin white flocculose layer. Sterile mycelium immersed in the substratum or closely appressed to the surface, fuscous. Conidiophores dilute fuscous, erect or bent, simple or apparently short-branched above because of the conspicuous sporogenous teeth, 1–3-septate, 18–39–(50) × 2.5–3.6 μ. Conidia hyaline, acrogenous or occasionally pleurogenous, mul-

tiseptate, the filament 1¼–2¼ times coiled, 3.6–4.5 μ thick, tapering toward the acutely rounded distal end, and toward the rounded, obliquely flattened basal end; diameter of coil 39.5–62 μ.

On moist decaying wood. Alabama.

The conidia of this species are attached obliquely, as are those of *H. roseus*, but from that species it may be distinguished by the erect fuscous and somewhat pellucid conidiophores that arise, for the most part, directly from the substratum, and not from hyaline creeping mycelium. The conidia of this species are also somewhat larger than are those of the related species.

Specimen examined:

United States:

Alabama: Montgomery, Oct. 1917, *R. P. Burke, 369.* TYPE. (Mo. Bot. Gard. Herb. 57236).

NOTE.—While this paper was in press, the writer in examining undetermined material, came across a specimen (Montgomery, Alabama, Aug. 1916, *R. P. Burke, 327*) which proves to agree with Morgan's description of the species, especially as regards the repent, anastomosing, fuscous conidiophores. The spore filaments, however, are 1.5–2 μ in diameter, and are coiled only 1½ to 2½ times. For the present, the specimen has been labelled *Helicomyces bellus* Morg.

EXPLANATION OF PLATE

PLATE 2

All drawings are made with the aid of a camera lucida. As reproduced they represent a magnification of × 500.

Figs. 1–3. *Helicoma Westoni* n. sp.

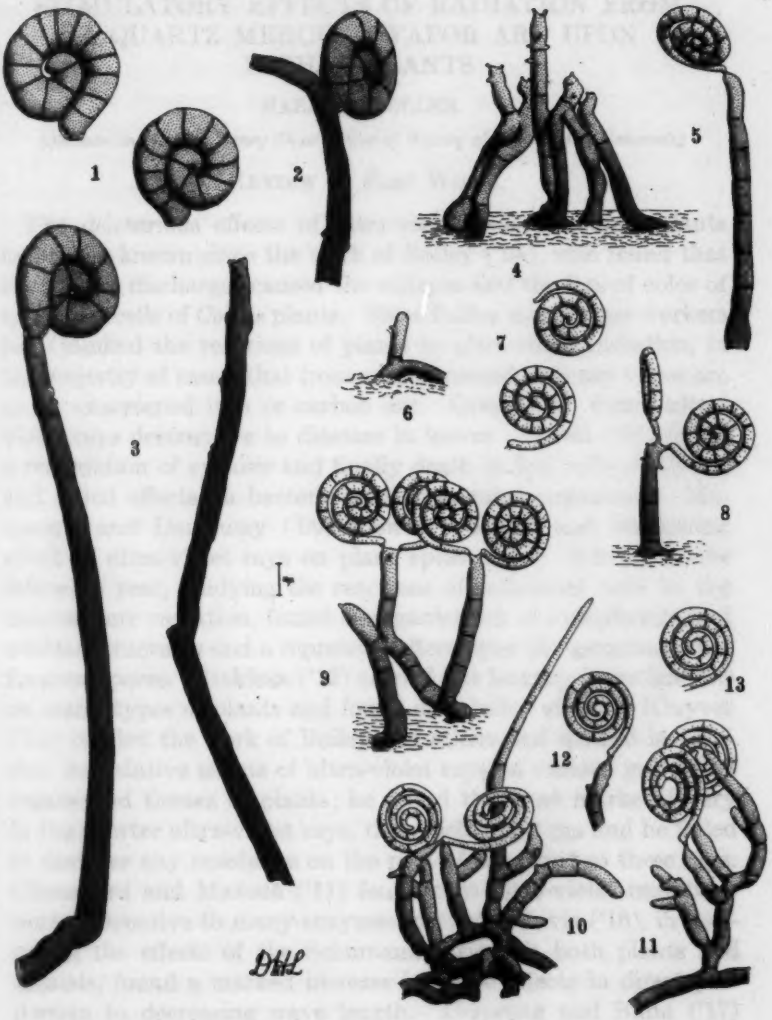
Spores and conidiophores. The characteristic hyaline collars may be discerned at the base of the conidia.

Figs. 4–9. *Helicoma anastomosans* n. sp.

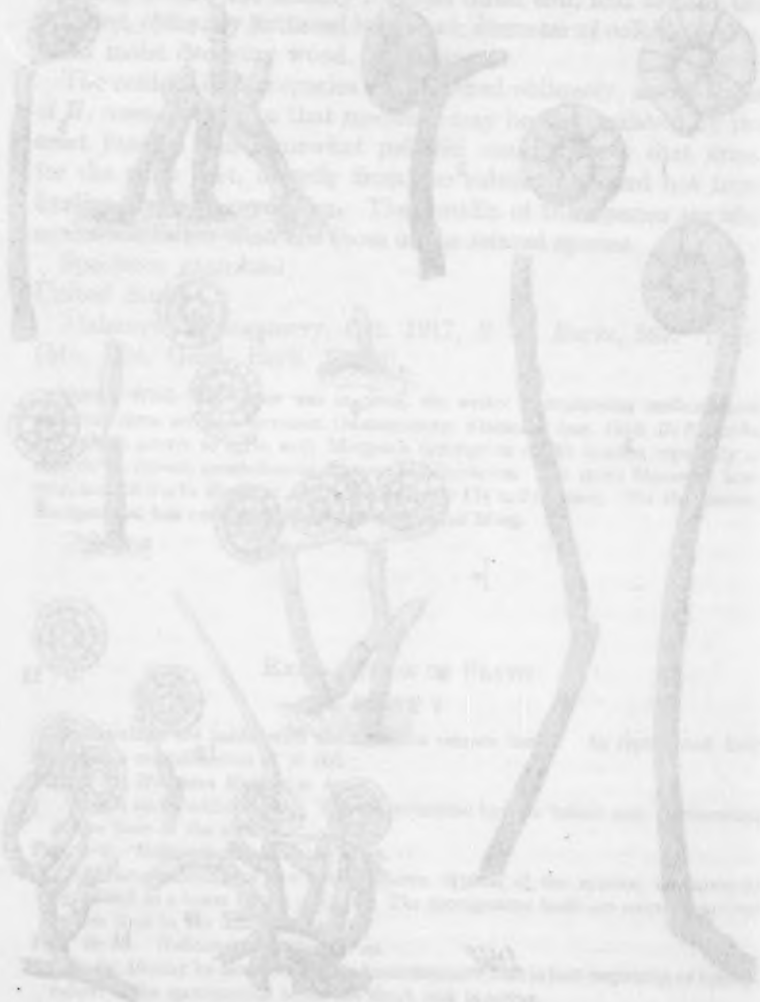
The anastomosing of the conidiophores, typical of the species, is shown in fig. 4, and to a lesser extent in fig. 5. The sporogenous teeth are more prominent in this than in the following species.

Figs. 10–13. *Helicoma tenuifilum* n. sp.

In fig. 10 may be seen an elongated conidiophore that is just beginning to branch below. The sporogenous teeth are short and tapering.



LINDER—HELICOSPOREAE



STIMULATORY EFFECTS OF RADIATION FROM A QUARTZ MERCURY VAPOR ARC UPON HIGHER PLANTS

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Review of Past Work

The deleterious effects of ultra-violet radiation upon plants have been known since the work of Bailey ('94), who found that electric arc discharges caused the collapse and the loss of color of epidermal cells of *Coleus* plants. Since Bailey many other workers have studied the reactions of plants to ultra-violet radiation, in the majority of cases, that from an unscreened mercury vapor arc or an unscreened iron or carbon arc. Green ('97) found ultra-violet rays destructive to diastase in leaves. Hertel ('05) found a retardation of cyclosis and finally death in leaf cells of *Elodea*, and lethal effects on bacteria and other micro-organisms. Maquenne and Demoussy ('09) showed a killing and blackening effect of ultra-violet rays on plant epidermises. Schulze in the following year, studying the reactions of individual cells to the mercury arc radiation, found disorganization of cytoplasmic and nuclear structures and a repressive effect upon the germination of fungous spores. Stoklasa ('12) carried out lengthy investigations on many types of plants and found only lethal effects. Kluyver ('11) verified the work of Bailey and others and studied in addition the relative effects of ultra-violet rays on various individual organs and tissues of plants; he found the most marked injury in the shorter ultra-violet rays, those below 290 mμ and he failed to discover any resistance on the part of the plant to these rays. Chauchard and Mazoué ('11) found that ultra-violet radiations were destructive to many enzymes in vitro. Bovie ('16), investigating the effects of the Schumann region on both plants and animals, found a marked increase in lethal effects in direct proportion to decreasing wave length. Ursprung and Blum ('17) used deplasmolysis as an indicator of injury and found that the wave-lengths below 290 mμ caused the greatest damage, that the presence of some pigments evidently increases the absorptive

capacity of cells for ultra-violet rays, but that chlorophyllous cells of the epidermis are more resistant than non-chlorophyllous ones.

Burge ('17), working on bacteria that liquefy gelatine, found that the rays injured the organisms not by destroying intracellular enzymes but rather by coagulating the protoplasm. In the next year, Schanz found that plants kept under Euphos glass, which screens out the ultra-violet spectrum, grew more rapidly and flowered earlier than plants which received even very small amounts of ultra-violet. Again Schanz ('19) found the maximum growth of plants in height occurred when the blue-violet portion of the spectrum was removed. Luers and Christoph ('23) studied further the injurious effects of ultra-violet upon yeasts, and in the same year Tanner and Ryder ('23) published results on similar experiments with yeasts; they found that the fermentative ability of the cells decreased in proportion to the length of radiation, that pigmented yeasts are more resistant to ultra-violet than colorless forms, and that there is evidently a relationship between cell size and the effects of radiation, since smaller yeast cells seemed more sensitive to injury than did larger ones. Coblentz and Fulton ('24) found that wave lengths extending from 365 mμ down through the Schumann region were bactericidal, the greater injury occurring in the shorter rays. Brooks ('26) studied the penetration of 2-, 6-dibromophenolindophenol into rayed cells of *Valonia* and found that the penetration of the dye was greater when the shorter waves were employed, which would seem to indicate that protoplasm loses its power of selective absorption under such treatment. Gibbs ('26), studying the effects of radiation from an unscreened mercury arc on *Spirogyra submaxima affinis* and *S. nitida affinis*, found that only rays of less than 3126 Ångstrom units appeared to be toxic. Russell and Russell ('27) rayed seedlings with an unscreened mercury arc and found that dwarfing resulted in direct proportion to the duration of exposure; they also found that the injurious effects were more marked in etiolated than in normal seedlings.

This is by no means all of the work which has been done upon the subject, but it is representative of the various aspects of the problem, and it illustrates all the toxic effects of ultra-violet radiation on plants, which may be summed up as follows:

1. Formative changes in the organism as a whole—e. g., the collapse of epidermal tissue, the burning off of hairs, the blackening of leaves, reduction in size of leaves, general dwarfing of the organism, etc.

2. Structural changes in the protoplasm: coagulation, bursting of cells, clumping of plastids, destruction of vacuoles, etc.

3. Changes in physiological processes: loss of selective absorption, cessation of cyclosis, aberrations of mitosis, destruction of enzymes, etc.

4. As the end result of the above-mentioned changes, the death of the organism.

That accelerative stimulation of plants can be brought about by ultra-violet radiation is another aspect of the subject which has received attention and which has proved extremely controversial in nature, in contrast to the subject of injurious effects. Numerous workers have reported varied types of stimulatory effects on both lower and higher plants—stimulated growth, increased production of food substances, of pigments, stimulated reproductive activity, etc.

Bonnier and Mangin ('86) reported a slight stimulatory effect of ultra-violet upon assimilatory processes in the plant. Tolomey ('94), using a magnesium light as a source of ultra-violet rays, found an increased formation of food substances in rayed plants. Grantz ('98) found that ultra-violet radiation caused an increase in the numbers of fruiting bodies produced by certain fungi. Laurent and Marchal ('03) reported that ultra-violet promoted the synthesis of proteins in plants. Stoklasa ('12) found that *Azotobacter* cultures, when rayed for very short periods—from 1 to 8 seconds—showed increased growth. Tsuji ('18) obtained increased growth and a higher percentage of sugar in sugar cane which was given weak doses of ultra-violet rays. Dufrenoy ('25) rayed zoospores of *Blephorospora* and *Phytophthora* and found that when the period of exposure was reduced to two minutes, the cilia were withdrawn within five minutes preparatory to germination, a process which normally requires several hours; when the dosage of ultra-violet was increased, only injury resulted. Euler ('25) obtained increased growth of the mycelium of *Penicillium glaucum* Link and *Rhizopus chinensis* Saïto by short exposures

to an unscreened arc, and he asserted that there is a certain optimal period of radiation for organisms, above which only injury occurs.

Coward ('27) reported an accelerated formation of vitamin A in the tissues of wheat seedlings under the influence of ultra-violet radiation; he found that this effect occurred only when rays shorter than 3130 Ångstrom units were removed. Sheard and Higgins ('27) found the shorter ultra-violet wave lengths to be stimulatory to the germination of seeds of cucumber and the longer wave lengths effective in promoting later growth of the seedlings. They explain the inhibitory effects of the rays ranging from 270 mμ to 320 mμ as being caused by the action of these rays in coagulating the seed albumen. They also state that the lesser wavelengths of light, especially those of the near or biologic ultra-violet, act as stimulative agents which modify the endogenous growth of the cells and of the organism, whereas the greater wave lengths of visible and infra-red rays influence the exogenous metabolic processes in the subsequent growth and development of the plant. Beeskow ('27) found that a ½-minute daily irradiation of soy-bean seedlings under an unscreened mercury vapor arc caused no injury and in some cases seemed to produce a slight stimulation to growth. Beeskow further discovered that rayed plants showed a slight increase in calcium and phosphorus content.

Nadson and Philippov ('28) reported that the longer ultra-violet rays stimulated the growth of several yeasts and mucors, while the shorter rays killed the organisms; in some of the fungi with which they worked, they found an increase in the numbers of reproductive organs produced, both asexual and sexual, under the influence of ultra-violet radiation. Stevens ('28) rayed cultures of *Coniothyrium* and *Glomerella* with a mercury vapor arc and found that the numbers of perithecia and pycnidia were greatly increased; in these fungi the reproductive organs are not normally produced until the cultures are very old, but under the influence of the ultra-violet radiation, they appeared almost immediately. This really cannot be considered as accelerative stimulation, since Klebs has shown that in many lower organisms, reproductive processes take place only when environmental con-

ditions become suddenly unfavorable for vegetative growth. The subject is by no means settled, however, and will bear further investigation. Stevens found only lethal effects on spores and mycelium with the unscreened lamp; the rays instrumental in bringing about the production of reproductive organs were those shorter than 313 mμ.

Delf, Ritson and Westbrook ('27), using an unscreened mercury arc, found injurious effects on a variety of plants, but found that when seedlings of some of them were rayed for 30 seconds daily, a small amount of increased growth was obtained; the number of plants used was small, however, and hence the results cannot be considered as of great reliability. McCrea ('28–'29) found that plants of *Digitalis purpurea* which were grown under vita-glass, transmitting to 289 mμ, in a greenhouse through the seedling stage, showed an increased digitalin content of 21 to 40 per cent, although there was no perceptible increase in the amount of growth of the plants. Eltinge ('28) rayed plants with a mercury arc, screened and unscreened, and found in some cases that growth was apparently stimulated when screens were used; she used a screen of vita-glass and one of quartz-lite glass; the former transmits to 289 mμ, the latter to 313 mμ; plants rayed with the vita-glass showed for the most part better growth than those rayed with the quartz-lite; both showed more growth than the controls; when the unscreened lamp was used, only injury occurred. Shortly after this work, Popp and Brown ('28) reported on experiments with some of the plants with which Miss Eltinge had worked; they found only injury under an unscreened lamp; moreover, they found that when the lamp was screened to give wave lengths down to only 300 mμ, no stimulation occurred, nor was there any injury. Fulton and Coblentz ('29) found indications of stimulation on moulds which they exposed to the mercury arc for short periods; when the periods of exposure were increased, injury resulted.

Newell and Arthur ('29) rayed tomato plants with a mercury arc, both unscreened and screened with filters transmitting small progressive portions of the ultra-violet spectrum, and found only injury in the rays shorter than the solar limit; the upper limit at which harmful effects were produced was found to be at 281.1

mμ; in the longer wave lengths (above 290 mμ) there was neither injury nor stimulation. Sheard, Higgins, and Foster ('30) reported the results of experiments on the germination and early growth of seedlings under various portions of the solar spectrum; their results indicated that the ultra-violet and infra-red portions of sunlight are stimulatory to germination and enhance growth and later development, but that they induce less chlorophyll formation than do other portions of the spectrum.

STATEMENT OF THE PROBLEM AND CRITICISMS OF PREVIOUS WORK

The object of this work is to determine whether or not radiation from a quartz mercury vapor air-cooled arc might cause definite accelerative stimulation in the growth of higher plants, in an endeavor to contribute something positive to the much-controverted subject. Certain criticisms of previous work may be offered which may be of aid in accounting for the discrepancies mentioned in the reviews. In the first place, most workers heretofore have neglected to furnish quantitative measurements of the radiant energy given off by the lamps with which they have worked; obviously differences in respect to this factor can be expected to account for a large portion of the disputed results. Secondly, accurate measurements of the wave lengths given off by the sources of radiation have been omitted in some cases; there can of course be no basis for the comparison of results obtained with a screened lamp transmitting to 300 mμ, with those obtained from an unscreened lamp which transmits, say, to 220 mμ, and yet very often attempts are made to correlate the findings of experiments conducted under such widely divergent conditions, with the result that endless and unnecessary disputes have arisen. In the third place, the methods of exposing the plants to the source of radiation have differed; some workers have rayed plants at a distance of 20 inches from the arc, still others at 100 inches, and so on; further, the periods of irradiation have varied widely, as well as the methods of applying them—some investigators have given the same dosage every day, others have increased the periods gradually throughout a series of daily irradiations, etc. In the last place, the experimental populations have in most cases been too small to make possible the exclusion of individual varia-

tion in interpreting the results; conclusions drawn from the reactions of six or eight plants cannot be of much value. This factor can be overcome only by the use of large numbers of individuals in the experiments; then, too, accurate statistical analyses have not been made of results, with the consequence that the reliability of measurements obtained has not been determined.

In the past, most workers have assumed the effects produced by the mercury and carbon and iron arcs to be due to the ultra-violet spectrum alone. It has been shown by Sheard and Higgins ('27) that the mercury vapor arc may give off as much as one third of its total radiation as infra-red. Hence, it is a flagrant disregarding of facts to assume that the effects of the mercury arc on organisms are due to the ultra-violet region alone. In this paper, the term "ultra-violet" is used to express this limitation—that is, to mean in reality, "the radiation from the mercury arc." In a continuation of the present work, the author intends to study the effects of the radiation from a lamp screened by a quartz water cell to remove the greater portion of the infra-red rays, upon the same plants used in this work.

In the prosecution of this work, an attempt has been made to reduce to a minimum the four objections raised in the second preceding paragraph.

Methods and Materials

The experimental methods used in this work were planned specifically in relation to three recent works on the subject of stimulation of plants by ultra-violet, that of Miss Eltinge ('28), of Popp and Brown ('28), and of Newell and Arthur ('29). Miss Eltinge reported that the radiation from a mercury arc, screened by vita-glass and quartz-lite, "was beneficial" to some of the plants with which she worked—*Cucumis sativus*, var. "Improved Green Hybrid," *Coleus Blumei*, *Bryophyllum pinnatum*, *Lactuca sativa*, and others. Popp and Brown, working on some of the same plants, reported only injurious effects with the unscreened lamp, and neither injury nor stimulation with the lamp screened to remove wave lengths below 300 mμ. Newell and Arthur likewise obtained only deleterious effects with the unscreened lamp in their work on tomatoes; above 281.1 mμ they found neither

injury nor stimulation. It was thought that certain differences in the experimental procedures of Miss Eltinge and of these other investigators might account, in part at least, for the apparently conflicting results, and so the methods they employed were carefully compared in order to devise a technique which might incorporate certain aspects of the methods of all three investigators and which thus might offer some common basis for comparison.

The following differences were noted:

1. Miss Eltinge rayed the plants she used for periods which began with 30 seconds on the first day and which increased by that same amount on each successive day. Popp and Brown, and Newell and Arthur used a constant period for each daily irradiation; no incremental method was used. The periods used by them varied from a few seconds to several hours.

2. Miss Eltinge used the vita and quartz-lite glass screens in her work; Newell and Arthur, and Popp and Brown used filters whose transmissions differed from those used by Miss Eltinge and in addition used the unscreened arc in attempting to find whether or not stimulation occurred.

3. Miss Eltinge rayed her plants at distances of 50 and 100 inches. Popp and Brown used a distance of 50 centimeters, Newell and Arthur a distance of 15 inches.

The experimental work described in this paper was planned upon the basis of these differences in the following manner:

Since the methods of irradiation employed by Miss Eltinge differed from those of Popp and Brown and Newell and Arthur, it was thought that, by using Miss Eltinge's procedures, which she reported to cause stimulation, on the plants employed by these other workers and reported by them to be unstimulated, it might perhaps be possible to accelerate their growth. Accordingly, the plants selected were *Cucumis sativus* L., var. "Early White Spine," used by Popp and Brown, and *Lycopersicum esculentum* Mill., the common tomato, which Newell and Arthur employed in their work. In addition to Miss Eltinge's method of applying the irradiation periods—that of daily increments of 30 seconds—, experiments using equal daily exposure periods were performed to determine whether or not the incremental method might enable the plants to become adjusted to the radiation and

thus to escape injury and perhaps even to derive some benefit from the gradually increased dosages. In order to make certain that any differences resulting from the two methods of dosage would be due only to the difference in the method and not to unequal quantities of energy received, the periods of exposure were planned so that at the end of the experiment the plants rayed according to the two procedures would have received exactly the same amount of radiant energy.

The source of ultra-violet radiation in these experiments was an air-cooled Uviarc quartz-mercury vapor arc from the Burdick Cabinet Co.; throughout the experiments the arc was used at 70 volts with a current of 6 amperes. In some of the work the lamp was unscreened, and in other portions the quartz-lite and vita-glass filters were used. Spectrographs showed that the unscreened lamp gave off radiation ranging from 578 mμ to 200 mμ; when the arc was screened with vita-glass, the ultra-violet spectrum below 289 mμ was removed; when the arc was covered with the quartz-lite filter, the rays below 313 mμ were removed.

In this work two experiments were performed, the first, a preliminary one, intended to "feel out" any tendencies which might become evident, the second, a more exhaustive investigation of the results obtained from the first. In the following discussion, these experiments will be designated as I and II respectively.

In experiment I, the plants were rayed at 50 inches for 4 weeks. The following experimental groups were used:

Set A—Controls.

Set B—Plants rayed, using a quartz-lite filter, for a period of 30 seconds on the first day, increased thereafter by an equal period daily.

Set C—Plants rayed, using a quartz-lite filter, for a period of 7.5 minutes daily.

Set D—Plants rayed with the unscreened arc, with radiation periods as in Set B.

Set E—Plants rayed with unscreened arc, the radiation periods as in Set C.

The groups in experiment I consisted of 15 plants each, a number probably too small to overcome the factor of natural variation in the final interpretation of results but nevertheless

large enough to indicate general trends. The plants were rayed daily, with the periods adjusted to insure equal amounts of energy for the rayed groups. In this experiment the plants were grown individually in 2-inch pots, in a mixture of three-fourths loam and one-fourth sand. The plants were moved about in the greenhouse at the end of each week to insure similar environmental conditions.

In experiment II the plants were rayed at 100 inches for 5 weeks. The experiment consisted of the following groups:

Set A—Controls.

Set B—Plants rayed, using a quartz-lite filter, for a period of 30 seconds on the first day, increased thereafter by 30 seconds daily.

Set C—Plants rayed, using a quartz-lite filter, for a period of 9 minutes daily.

Set D—Plants rayed, using a vita-glass filter, with irradiation periods as in Set B.

Set E—Plants rayed, using a vita-glass filter, with irradiation periods as in Set C.

Each group in experiment II consisted of 100 plants, a number large enough to reduce to a minimum the factor of individual variation.

The heights and numbers of leaves in the plants in both experiments were recorded at the beginning of the experiments, at the end of half the period, and again at the conclusion. In addition, in experiment II, wet weights, dry weights, and ash weights were determined, and from these results the dry weight percentages of wet weight and the ash-weight percentages of dry weight were calculated. In the determination of dry weights, the plants were dried in an oven at 60° C. After the weighings had been completed, the plants were incinerated in porcelain crucibles in a Bunsen flame until the ash fused; the covered crucibles were then placed in a desiccator to cool, in order to exclude the possibility of error from the condensation of atmospheric water vapor upon the ash or crucible. Since the time was not available for making 1,000 individual ash determinations of the plants in experiment II, 30 plants were selected from the control set and 30 from the group which showed the greatest growth under the arc, 10 plants

from among those which showed growth greater than that of the group average, 10 from those which showed growth equal to that of the group average, and 10 from those which showed less growth than the group average.

Intensity measurements were made by means of a Leeds and Northrup high sensitivity type P reflecting galvanometer #2239, with a sensitivity of .7 microamperes, and two Cenco linear thermopiles. A carbon filament incandescent lamp, from the Bureau of Standards of the U. S. Department of Commerce, standardized to give a radiation of 86.2×10^{-8} watts per square millimeter of receiving surface at two meters when lighted at .4 amperes and 99.5 volts, was used as a basis for computing the radiant energy given off by the arc. The intensity measurements are as follows:

At 100 inches:

Unscreened arc—956.44×10^{-8} watts per sq. mm.
Vita-glass —732.70×10^{-8} watts per sq. mm.
Quartz-lite —724.08×10^{-8} watts per sq. mm.

At 50 inches:

Unscreened arc—3825.76×10^{-8} watts per sq. mm.
Vita-glass —2930.80×10^{-8} watts per sq. mm.
Quartz-lite —2896.32×10^{-8} watts per sq. mm.

Observations and Results

Experiment I

Cucumbers.—The first visible effects on rayed plants appeared in set E, rayed 7.5 minutes daily with the unscreened arc, at the end of a week's period of irradiation. The upper epidermis appeared shiny and there was a slight curling of the younger leaves. Upon examination with a hand lens, it was found that the hairs on the upper epidermis had been completely burned off. These effects rapidly became intensified; after about 12 days the enlargement of young leaves had ceased entirely and all of the leaves of the plant were badly curled. The leaves were stiff and brittle, and showed a slight brownish discoloration of the upper surfaces. At the end of 22 days the plants had practically ceased growing, and death followed a few days later.

In set D, rayed for incremental periods with the unscreened arc, the first manifestations of injury were not as pronounced as

those in set E. The first effects of burning became noticeable on about the twelfth day and became gradually more intense, culminating in death on about the twenty-eighth day. At the time of death, the leaves were somewhat larger and more numerous than those in set E and the plants were somewhat taller. The growth differences are shown in table 1 at the end of this section.

There were no striking differences between sets A, controls, and B, rayed with the quartz-lite filter for incremental periods, at any time during the experiment, except that some plants in set B were slightly taller than those in set A. Since 9 of the 15 plants in set B were taller than the tallest plants in set A, it seemed that some small amount of stimulation of growth had occurred in set B, but, as has been stated before, the number of plants was not large enough to overcome individual variation. Hence, no definite interpretation can be placed upon the results. The number of leaves in set B was slightly greater than that in set A.

Set C, rayed 7.5 minutes daily with the quartz-lite filter, showed a perceptibly slower rate of growth and a smaller number of leaves than set B. Aside from this, there were no differences between the two sets. Neither showed any injury whatsoever, and the leaf sizes were approximately equal.

It will be seen from the figures in table 1 that the growth rate in set A during the first two weeks was slightly less than that occurring during the last two weeks of the experiment. In set B the same relationship held, but in set C the growth rate through the last two weeks was less than that of the first two weeks; this condition prevailed in sets D and E also. It is interesting to note that these growth relations were practically identical in the tomato plants.

Tomatoes.—The various experimental groups of tomatoes stood in approximately the same relation to each other as did the cucumber groups. Sets D and E showed the same type of injury as did the cucumbers—burning off of epidermal hairs, discoloration of epidermal tissue, the final cessation of growth, and the death of the plant. The tomatoes seemed slightly more sensitive in their reaction to the unscreened arc than did the cucumbers, for they exhibited signs of injury after about 6 days of irradiation. They ceased growing at about the same time as did the cucumbers,

but they remained alive a few days longer. The injurious effects were less pronounced in set D than in set E, as was also the case in the cucumbers.

The plants in set B showed a slightly increased amount of growth over the controls, and those in set C showed less growth than did set B. Aside from this, there were no differences in the plants in these groups. The growth rates in sets A and B were greater during the last two weeks of the period than during the first two; in sets C, D, and E, the reverse occurred. The similarity of these reactions in both cucumbers and tomatoes seemed to indicate a tendency—that of the repression of growth by ultra-violet radiation when the dosage exceeds an optimum value. This will be discussed later in the paper.

Plate 3, fig. 1, shows the appearance of plants from the five sets of tomatoes at the end of the four weeks of exposure.

TABLE I

CUCUMBERS

Set	Average increase in height of plant and in number of leaves during experiment					
	1st 2 weeks		2nd 2 weeks		4 weeks—total	
	Height	Leaves	Height	Leaves	Height	Leaves
	cm.		cm.		cm.	
A	4.71	2.85	4.92	1.00	9.63	3.85
B	4.64	2.57	5.30	2.21	9.94	4.87
C	4.23	2.57	3.44	1.33	7.67	3.80
D	4.02	2.06	.94	.71	4.96	2.77
E	3.05	2.01	.17	0.00	3.22	2.01

TABLE II

TOMATOES

Set	Average increase in height of plant and in number of leaves during experiment					
	1st 2 weeks		2nd 2 weeks		4 weeks—total	
	Height	Leaves	Height	Leaves	Height	Leaves
	cm.		cm.		cm.	
A	3.99	1.20	6.33	2.00	10.32	3.20
B	4.76	1.87	6.27	1.22	11.03	3.09
C	3.86	2.00	3.20	1.30	7.06	3.30
D	3.02	1.28	1.82	.54	4.84	1.82
E	2.44	1.25	1.25	.40	3.69	1.65

EXPERIMENT II

When indications of stimulation were found in the plants of experiment I, rayed through the quartz-lite filter, it was decided to perform another experiment to study further these stimulatory effects and in addition to study with the aid of the vita-glass filter the effects of the rays between 313 mμ (the quartz-lite limit) and 289 mμ. To overcome errors due to natural variation, the number of plants in each group was increased to 100; the plants were grown in large-sized greenhouse flats, 25 in a flat, in the same soil as was used for experiment I. The following were the experimental groups:

Set A—Controls.

Set B—Quartz-lite filter; rayed 30 seconds the first day and 30 seconds additional on each following day.

Set C—Same as set B, but rayed 9 minutes daily.

Set D—Vita-glass filter; rayed as in set B.

Set E—Vita-glass filter; rayed as in set C.

The experiment was carried through 5 weeks; the plants were rayed at 100 inches. Statistical analyses were made of the results of experiment II to determine their reliability.

Cucumbers.—The growth increases and the various weights are shown in tables IIIa and IIIb. The plants at the beginning of the experiment averaged about 5 cm. in height.

As the figures show, there was not much difference among sets A, B, C, and D, as to growth rate and number of leaves produced. In set E, however, the increase in elongation during the five weeks was significantly greater than that of the controls, about 35 per cent greater. The number of leaves produced in set E was also larger than that of the controls. Aside from these factors, there were no other apparent differences between sets A and E—leaves were of approximately the same size and the numbers of flowers produced in both groups were about equal. There were no evidences of injury in any of the rayed plants. The results of this part of the experiment are shown in plate 3, fig. 2.

Tomatoes.—The tomatoes at the beginning of the experiment averaged about 2.5 cm. in height. The results of the experiment are shown in table IVa and IVb, and in plate 3, fig. 3.

Here, as in the cucumbers, there were no great differences among sets A, B, C and D, although in general the rayed sets showed

slightly more growth than the controls, and furthermore the wet and dry weights and the dry percentages of wet weights were slightly greater in the rayed sets. In set E, the growth in height was very definitely greater, by approximately 35 per cent than in the controls. The number of leaves produced in set E was greater than that in set A, and the wet and dry weights and dry-weight percentage of wet were considerably larger. There were no signs of injury in the rayed plants.

In the tomatoes and cucumbers, the rayed sets showed a slightly greater dry-weight percentage and ash-weight percentage. Furthermore, in both plants in experiment II, growth was greater in all of the sets during the last 2½ weeks than during the first 2½. This would seem to indicate that the limit at which there would be repression of growth and injury by ultra-violet radiation had not been reached. The effects of passing that limit are shown by the results of experiment I.

TABLE IIIa

CUCUMBERS

Set	Average increase in height of plant and in number of leaves during experiment					
	1st 2½ weeks		2nd 2½ weeks		5 weeks—total	
	Height	Leaves	Height	Leaves	Height	Leaves
	cm.		cm.		cm.	
A	5.72	2.86	18.54	4.36	24.26	7.22
B	5.25	3.31	22.06	4.41	26.31	7.72
C	5.16	2.87	17.79	3.64	22.95	6.51
D	4.80	2.93	16.26	3.81	21.06	6.74
E	6.18	3.08	26.88	5.61	33.06	8.69

TABLE IIIb

CUCUMBERS

Set	Weights		
	Average wet weight	Average dry % of wet wt.	Average ash % of dry wt.
	gms.		
A	11.75	9.01	18.02
B	11.98	10.01	
C	11.92	9.92	
D	12.02	10.08	
E	14.16	9.98	20.29

TABLE IVa

TOMATOES

Set	Average increase in height of plant and in number of leaves during experiment					
	1st 2½ weeks		2nd 2½ weeks		5 weeks—total	
	Height	Leaves	Height	Leaves	Height	Leaves
	cm.		cm.		cm.	
A	5.91	4.50	13.97	.56	19.88	5.06
B	5.55	4.79	16.12	.80	21.72	5.59
C	5.17	4.62	18.00	1.00	23.17	4.72
D	4.90	3.99	16.81	1.61	21.71	5.60
E	5.67	3.92	21.23	2.19	26.90	6.11

TABLE IVb

TOMATOES

Set	Weights		
	Average wet weight	Average dry % of wet wt.	Average ash % of dry wt.
	gms.		
A	10.52	8.07	16.98
B	11.34	9.65	
C	13.06	9.77	
D	12.44	10.02	
E	15.18	10.45	19.15

Statistical analyses of the increases in length, the dry-weight percentages of wet weights, and the ash-weight percentages of dry weight were made. A $\frac{\text{mean difference}}{\text{probable error diff.}}$ of 4.00 is accepted as indicating complete reliability of results.[1] The values of the analyses are shown below:

[1] Garrett, H. E. Statistics in psychology and education. p. 136. London, 1926.

CUCUMBERS

Set	Height cm.	Dry % of wet wt.	Ash % of dry wt.
$\frac{A}{B}$	18.08	10.14	
$\frac{A}{C}$	32.82	7.03	
$\frac{A}{D}$	13.95	9.29	
$\frac{A}{E}$	54.03	13.65	9.18

TOMATOES

Set	Height cm.	Dry % of wet wt.	Ash % of dry wt.
$\frac{A}{B}$	5.50	5.01	
$\frac{A}{C}$	12.36	5.98	
$\frac{A}{D}$	9.34	9.76	
$\frac{A}{E}$	55.02	18.89	7.15

Discussion

The results of these experiments demonstrate that at least the longer ultra-violet wave lengths—those of the ultra-violet solar spectrum—produce accelerated growth in higher plants when applied in sufficient dosage. The results further show the lethal effects of the shorter wave lengths on the same plants.

Several interesting facts were brought out by the work. The greater stimulation at 100 inches as compared with that at 50 inches is in accord with earlier findings (Eltinge, '28), and may be attributed to the fact that at 50 inches some of the shorter rays, which may pass through the filter and which may be slightly repressive though not destructive to growth processes, reach the plants. At 100 inches, most of these short rays are screened out

or are so diminished in intensity by the atmosphere of the increased distance through which they must pass that they exert none of their inhibitory effects. On the other hand, the differences in effects at the two distances may be merely a function of differences in amounts of radiation received, assuming a stimulatory limit, above which increased radiant energy produces only retardation of growth, or even pronounced injury. The radiation at 50 inches, even if qualitatively about the same as it is at 100 inches, is four times more intense than at 100 inches; hence it is logical to assume that if the radiation at 100 inches is of the proper intensity to induce a high degree of stimulation, the intensity at 50 inches, being, as it were, of four times greater energy value, closely approaches or surpasses slightly the limit of beneficial influences and produces less stimulation than at 100 inches, no stimulation at all, or at the other extreme, retardation.

The use of incremental and constant periods of radiation produced varied and somewhat uninterpretable results. In experiment I, the plants rayed with increments (B—screened, D—unscreened) showed better growth than those rayed for constant daily periods (C—screened, E—unscreened). In set B the growth rate was greater than that of the controls; in set C, the growth rate was less than those of the controls and of set B. At the end of the irradiation period, the plants in set D were taller and the leaves were slightly larger than those in set E; furthermore, the D plants lived a few days longer than did the E plants before succumbing to the lethal action of the ultra-violet radiation.

These differences cannot be accounted for on the basis of different amounts of energy received, since the periods were adjusted to insure equal energy values for all groups in the experiment. Hence the explanation seems to lie in the building up of a resistance in the plants rayed incrementally by means of a gradual increase in dosage, an "accustoming" process, as it were. Since the B plants showed more stimulation, the incremental process (when the lamp is screened) would seem to consist of two reactions: gradual adjustment of the plant to the radiation, followed by accelerated growth. When the lamp is unscreened, the incremental radiation reduces the injury to the plants. The failure of the constant-period method to induce more rapid growth in the

case of the screened lamp may perhaps be due to the fact that without the adjustment process the dosage at the beginning is above the beneficial limit and hence only negative results occur.

The effect of the incremental method, on the other hand, might be explained upon this basis: that during the latter half of the five-weeks radiation period, the plants which were rayed by the incremental method were receiving considerably more energy per day than were those in the constant-period groups; this greater energy coming at a time when the growth rate was rapid may have caused the greater stimulation. This explanation seems to be invalidated, however, by the experiments in which the plants were rayed with the unscreened arc; here during the latter half of the five-weeks period, the plants in the incremental group were likewise receiving more energy per day than those in the constant-period group. If the above explanation were the true one, it would be expected that the plants in the incremental group would show the greater injury, but, as a matter of fact, the plants rayed incrementally showed *less* injury. This would seem to indicate that the resistance theory is more satisfactory.

The necessity of using an incremental method at 50 inches to produce any stimulation whatsoever might explain the negative results of Popp and Brown and of Newell and Arthur in their attempts to discover stimulation in the wave lengths above 300 mμ, since they both worked at distances of less than 50 inches—Popp and Brown at 50 cm., Newell and Arthur at 15 inches.

These explanations, however logically they coincide with the results of experiment I, are not wholly satisfactory when they are applied to experiment II. In the cucumber plants rayed through the quartz-lite filter, the incremental method induced greater growth than did the constant-period method. In all other rayed sets in experiment II, however, both in cucumbers and tomatoes, the reverse was true—the plants rayed incrementally showed less growth than those rayed for constant periods. An explanation of this is wanting. One possible cause—but hardly an important one—may be the fact that the plants used in experiment I were younger than those in experiment II, and that there may be different relations in the adjustment reactions of plants at different

periods in their early development. Another suggestion to explain this variation is difference in wave length. In experiment I, where the increment sets showed the greater growth, the vita-glass filter was not used, but instead, the quartz-lite and the unscreened arc. In experiment II, in the cucumbers, the increment quartz-lite set showed greater growth than the constant-period quartz-lite set; in the tomatoes the reverse was true, but the difference was slight. In both tomatoes and cucumbers rayed through the vita-glass, however, the incremental method showed much less growth than the constant-period method. Hence, it appears as though the quality of the spectrum transmitted by the vita-glass might have caused this variation from conditions in experiment I.

It is interesting to note that not only the growth rates and numbers of leaves produced in rayed plants were greater than in the controls, but that also the dry-weight and ash-weight proportions were greater. The fact that the ash content of the rayed plants showed an increase over the controls is especially interesting, since ultra-violet radiation has been shown also to increase the mineral content, especially the calcium and phosphorus content, of animal tissues in the case of rickets and other deficiency diseases (Kramer and Boone, '22; Orr, Holt, Wilkins and Boone, '23; Ellis and Wells, '25). Beeskow ('27) reported that ultra-violet radiation increases the calcium and phosphorus content of soybeans which are exposed to a mercury arc.

Since greater stimulation occurred under the vita-glass filter than under the quartz-lite, it seems that wave-lengths between 313 mμ and 289 mμ are more potent in inducing growth than those longer than 313 mμ. It might be argued that the difference in stimulation produced by the two filters is a function of varying intensities of the radiation which they transmit; however, the intensity measurements show such slight differences that this argument is seemingly not valid. This agrees with the general findings concerning this shorter portion of the solar spectrum—its greater activity in photochemical processes, its greater efficiency in the treatment of rickets, etc.

Summary

1. The longer ultra-violet wave lengths under certain conditions described in this paper are stimulating to the growth of higher plants.

2. The injurious effects of the short wave lengths have been again demonstrated.

3. Dry weight and ash weight of plants employed in this work increase with ultra-violet treatment.

4. Wave lengths between 313 mμ and 289 mμ produce greater stimulation than those longer than 313 mμ.

5. The incremental method for the most part produces greater growth than the constant-period method, indicating an induced adjustment of the plants to the gradual increase of dosage.

6. The more marked stimulation occurs at a greater distance than that used by most other workers.

7. Statistical analyses proved the reliability of the results.

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Explanation of Plate

PLATE 3

Fig. 1—Tomatoes—Exp. I

A—Control.
B—Rayed with quartz-lite filter by incremental method.
C—Rayed with quartz-lite filter by constant-period method.
D—Rayed with unscreened arc by incremental method.
E—Rayed with unscreened arc by constant-period method.

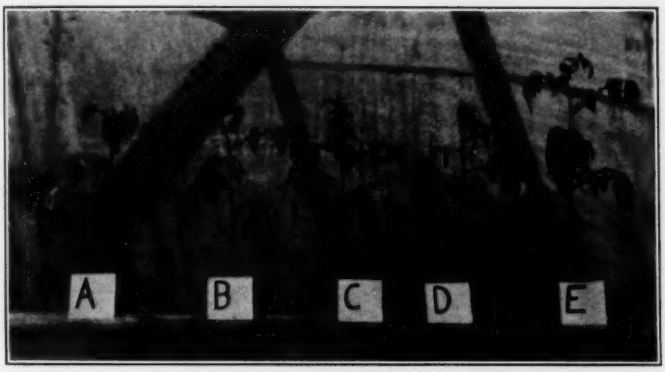
Fig. 2—Cucumbers—Exp. II

A—Control.
B—Rayed with quartz-lite filter by incremental method.
C—Rayed with quartz-lite filter by constant-period method.
D—Rayed with vita-glass filter by incremental method.
E—Rayed with vita-glass filter by constant-period method.

Fig. 3—Tomatoes—Exp. II

A—Control.
B—Rayed with quartz-lite filter by incremental method.
C—Rayed with quartz-lite filter by constant-period method.
D—Rayed with vita-glass filter by incremental method.
E—Rayed with vita-glass filter by constant-period method.

1



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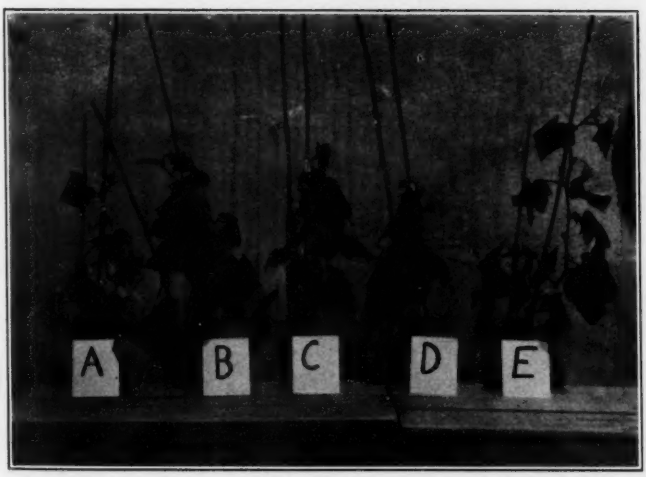

3

FULLER—EFFECTS OF ULTRA-VIOLET RADIATION

A STUDY OF PLANT DISTRIBUTION IN RELATION TO THE ACIDITY OF VARIOUS SOILS IN MISSOURI

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The various kinds of electrometric and colorimetric methods that have been used to determine hydrogen-ion concentration are all relatively recent. Böttger's work in 1897 on the determination of the neutral point in titrating acids with alkalis by use of a gas chain marked the introduction of electrometric methods, and served as an impetus for subsequent workers, such as Hildebrand, Cumming and Gilchrist, Hasselbalch, Clark, Michaelis, Walpole, and others, each of whom helped to perfect the electrometric method. In 1914 the electrometric method was first applied to the measurement of the hydrogen-ion concentration of soil suspensions by Fischer ('14) in Germany, and subsequently in America by Hoagland and Sharp ('18), and by Gillespie ('16) and his co-workers.

In 1909 Sørensen introduced the colorimetric methods, which were later improved and applied to biological fluids by him, Palitzsch, and Walpole. Further improvements were made by Clark and Lubs ('17) in the use of a different set of indicators and buffer mixtures. Biilmann and Lund, in 1921, showed that with quinhydrone it was possible to form an electrode capable of being used for hydrogen-ion determinations, and in 1923 he ('24) applied this electrode in the determination of the hydrogen-ion concentration of soils. The results compared favorably with those of the hydrogen-electrode method. Before Biilmann's discovery the latter method had proven the most satisfactory electrometric method. However, in recent years the quinhydrone electrode has steadily increased in use, and at present seems for various reasons to be superior to the hydrogen electrode. Bayer's ('26) application of this electrode to soil studies has been followed by a number of other workers. The Report of the Committee on Soil Measurements ('30) of the International Society of Soil Science on the "Results of comparative investigations on the quinhydrone electrode method" shows definitely that this method is at present the most satisfactory one.

In the studies reported here a uniform procedure was followed. Soil samples collected on one day were tested in the laboratory on the day following. In practically all cases a 1:1 soil-water ratio was used, being in most instances 500 grams of soil to 500 cc. of water. The mixture of soil and water was shaken violently for approximately one minute, and subsequently allowed to stand an hour. The supernatant liquid was then poured out, and readings made, four or five of which were taken in almost every determination, so that reliable results might be obtained. After each reading the test-tube vessel and electrode were well rinsed with tap and distilled water. Altogether, twenty-four different soil samples were tested. Only surface soils, taken from a depth of about six inches, were used. For most determinations the samples were collected from areas with typical or conspicuous associations rather than from isolated places exhibiting unusual plants. Seven sets of samples were taken. These comprised two sets from Tilsit soil localities, two from two Hagerstown soil localities, one from a Union soil area, one from the so-called "Rough stony land" area, and a final set from a locality possessing Clarksville stony loam soils. The various names given to these soils are those adopted by the Soil Survey of Missouri.

TILSIT SOILS

The belt of Tilsit, like all the belts along the eastern border of the Ozark dome, is very narrow. The Tilsit soils in Jefferson County are derived from the Crystal City or St. Peter sandstone, which is gray to white, and composed of extremely well-rounded, transparent, coarse quartz grains held together very loosely by a small amount of calcareous cement. This sandstone is subject to severe erosion, and deep gorge-like areas and cliff faces are not uncommon.

Set A.—Three samples were obtained on April 14, 1929, along the high sandstone bluffs back from the Meramec River about five miles southeast of Pacific, Jefferson County, Missouri.

Sample 1. This was obtained on the sandstone bluffs, about five feet from the base. A number of plants of *Lycopodium lucidulum* Michx. were growing on a substratum of *Polytrichum commune* L., mats of which grew on the bare sandstone rock. The pH value of the sample was 4.898.

Sample 2. This was taken at the base of the bluffs where the soil was very sandy, on level ground, in shade. A large colony of *Mertensia virginica* (L.) Link grew here. The soil was slightly subalkaline, having a pH of 8.278.

Sample 3. This soil was taken about thirty feet from the base of the bluffs, and was dark brown and not as sandy as that of the previous sample. It came from near the top of a small slope bordering a stream outlet, where *Dicentra Cucullaria* (L.) Bernh. and *D. canadensis* (Goldie) Walp. grew profusely in dense shade. The soil was circumneutral, being pH 6.588. This is one of the few localities in Missouri for *Dicentra canadensis* (Goldie) Walp.

Set B.—Five samples were obtained on May 12, 1929, about six miles southeast of Catawissa, Missouri. In this region occurred the same formation of sandstone as in Set A, which was approximately six miles north.

Sample 1. This was collected on one of the sandstone bluffs that bordered a ravine. The sample was taken about ten feet from the base of the bluff above a spring, where the sandstone was very loose and crumbly. Growing in abundance were *Sullivantia renifolia* Rosendahl (a species never before reported from Missouri), *Hydrangea arborescens* L., and *Marchantia polymorpha* L. The sample was circumneutral, the pH being 7.65.

Sample 2. This was taken on top of a badly weathered sandstone glade, where the rock was exposed and steeply sloping on the brink of the ravine. The sandy soil was thin and scattered, and never reached a depth of over a few inches. The exposure was dry and sunny. *Talinum teretifolium* Pursh and *Polytrichum commune* L. were the chief plants found. On the day the soil was collected a stream, caused by recent rains, was rushing swiftly down the outcrop near the plants, and was washing away much of the soil. The pH of the soil was 5.85.

Sample 3. This was collected in the floor of a deep ravine bordered by high sandstone bluffs. A shallow stream flowed through the valley. There was a rich deciduous tree growth, which shaded the ground plants. The soil was dark brown to slightly black, quite rich, and contained a fair percentage of sand. On the area from which the sample was taken grew *Orchis spectabilis* L., *Corallorrhiza maculata* Raf., and in the immediate envi-

rons was found *Aplectrum hyemale* (Muhl.) Torr. The soil was neutral, its pH being 7.2. The occurrence here of *Orchis spectabilis* L. is to be noted especially, as it is one of the rarest of Missouri plants.

Sample 4. This was procured from the same valley as Sample 3, but nearer the sandstone bluff and nearer the stream, where the soil was sandier. *Orchis spectabilis* L., *Panax quinquefolium* L., and *Smilacina racemosa* (L.) Desf. grew here. The soil was slightly subalkaline, the pH being 8.26.

Sample 5. This sample was taken above a sandstone ravine, in a situation similar to that of sample 2, but at a lower level and at a spot where more soil had accumulated. The exposure was a sunny, mossy slope above a ravine. Here grew an abundance of *Krigia Dandelion* (L.) Nutt. and *Tradescantia bracteata* Small. The soil was very sandy, and of a yellowish brown hue. The pH of the sample was 5.89.

HAGERSTOWN SOILS

The belt of the Hagerstown series, which occurs along the eastern border of the Ozark dome, like that of Tilsit soils, is very narrow. The soil is derived from the Trenton limestone of middle Ordivician age. The rocks are usually chert-free, finely crystalline, rather hard and compact, and of a dark gray color. In Jefferson County the topography in the Hagerstown belt is rough, and considerable areas of limestone glades occur more or less overgrown with *Juniperus virginiana* L.

Set A.—Five samples were collected on May 5, 1929.

Sample 1. This was collected between Imperial and Seckmann, Missouri, on limestone bluffs which were exposed to the sun and subject to drought. The sample was from cracks or narrow ledges on the rock where a small amount of soil had accumulated. A great abundance of *Cheilanthes Feei* Moore was observed. Plants associated with it were *Aquilegia canadensis* L., *Hydrangea arborescens* L., and *Heuchera hirsuticaulis* (Wheelock) Rydb. The soil was slightly subalkaline, having a pH of 8.482.

Sample 2. This was taken between Imperial and Seckmann, Missouri, in wet soil, in an open valley exposed to the sun and about 30 feet from a road. The soil of the valley floor probably received some alluvial deposits from Rock Creek, a stream about

150 yards distant. It may have also received material from the high limestone hills about 15 yards away, in the opposite direction from that of the stream. The plants found growing in this soil were *Acorus Calamus* L., *Typha latifolia* L., and *Mentha spicata* L. The pH of the soil was 7.285.

Sample 3. This was taken about two miles southwest of Glen Park, Missouri, at the head of a valley leading into a ravine. A stream flowed near by. The woods were dominated by oaks, chiefly *Quercus alba* L. and *Quercus rubra* L. The ground plants growing here were *Erigeron pulchellus* Michx., *Krigia amplexicaulis* Nutt., *Tradescantia bracteata* Small, *Phlox divaricata* L. with a rose-red corolla, and *Cornus florida* L. The soil was free from stones, and had a pH of 6.826.

Sample 4. This was collected about 100 yards from the previous sample, in a dry, mossy, sunny thicket, bordering on open oak woods, near the lower portion of the hill. *Castilleja coccinea* (L.) Spreng. in abundance, *Pedicularis canadensis* L., *Heuchera hirsuticaulis* (Wheelock) Rydb., *Geranium maculatum* L., *Erigeron pulchellus* Michx., *Krigia amplexicaulis* Nutt., *Ranunculus fascicularis* Muhl., and *Polystichum acrostichoides* (Michx.) Schott occurred here. The soil was a light brown clay, stone-free, and was subacid, having a pH of 5.806.

Sample 5. This was collected about one mile northwest of Barnhart, Missouri, near the top of a high, cherty limestone hillside, with a southern exposure, and consequently dry and exposed to the sun. The locality was a glade type, and bordered on a thicket of post oak and black-jack oak. The plants growing here were *Monarda Bradburiana* Beck, *Zizia aurea* (L.) Koch, *Brauneria angustifolia* (DC.) Heller, and *Parthenium integrifolium* L. The pH of the soil was 7.438.

Set B.—There were five samples of soil included in this second set, collected on May 20, 1929. The area was six miles west-southwest of Pevely, and approximately six miles distant from that from which the previous soil samples were taken. This country has considerable areas of limestone glades grown over with *Juniperus virginiana* L.

Sample 1. This was obtained halfway up a limestone hill. The surface limestone was broken into fragments, leaving exposed a

bare rocky glade, with sunny exposure, bordered by cedar trees. *Oxalis violacea* L., *Brauneria angustifolia* (DC.) Heller, *Houstonia longifolia* Gaertn., *Viola pedata* L., *Agave virginica* L., and *Psoralea tenuiflora* Pursh were found here. The soil was subalkaline, its pH being 8.448.

Sample 2. This was collected in rich limestone woods, about halfway up a steep, wooded and densely shaded hill. *Tilia americana* L., *Acer saccharum* Marsh., *Cornus florida* L., *Carya cordiformis* (Wang.) K. Koch, *Ulmus americana* L., and several species of *Quercus* were growing here. Towards the base, massive limestone outcrops occurred. A sample of soil was taken near a huge limestone boulder, where there grew several plants of *Aquilegia canadensis* L. and *Cystopteris bulbifera* L. The soil was dark brown in color and slightly alkaline, its pH being 8.363.

Sample 3. This was taken on the floor of a limestone ravine, near a stream. There was a heavy growth of *Acer saccharum* Marsh., *Ulmus americana* L., *Benzoin aestivale* (L.) Nees, *Aesculus glabra* Willd., and *Quercus alba* L., which shaded the ground plants. *Corallorrhiza maculata* Raf., *Viola striata* Ait., and *Botrychium virginianum* (L.) Sw. were in the immediate vicinity. The soil was a rich stony loam with much humus, and of a dark brown color. It was found to be slightly subalkaline, having a pH of 8.227.

Sample 4. This sample was dug about one-third the way up a thinly shaded hill covered chiefly with several species of *Quercus*, some *Cornus florida* L., and a few species of *Carya*. The soil was dark brown, thickly covered in most places with oak leaves, and was, on the whole, stone-free. The plants found growing here were *Rosa humilis* Marsh., *Antennaria plantaginifolia* (L.) Richards., *Rubus occidentalis* L., *Vaccinium vacillans* Kalm, and *Cunila origanoides* (L.) Britton. The soil was neutral, having a pH of 7.081.

Sample 5. This was collected in soil full of fragments of chert and pure limestone, at the base of a hill covered with oak and hickory. The spot was located just above the bank of a stream and opposite the hill from which the previous sample was collected. The woods here were rather open. The plants found were *Baptisia bracteata* (Muhl.) Ell., *Viola pedata* L., *Monarda Bradburiana*

Beck, *Antennaria plantaginifolia* (L.) Richards., and *Polygonatum commutatum* (R. & S.) Dietr. The soil was of a reddish-brown color, stony, and argillaceous. It was found to be subacid, and its pH 5.89.

UNION SOILS

This comprised a set of soils gathered at Gray Summit, Franklin County, Missouri. The rocks from which the Union soils are derived are the Jefferson City or Beekmantown limestones, of lower Ordivician time; these rocks are a series of moderately cherty, argillaceous, and more or less shaly and thinly bedded limestones. The topography in Franklin County where the Union soils occur is rather rough. All of the samples were collected on slopes where the soil was very shallow and the bedrock was exposed, making limestone glades. *Juniperus virginiana* L. and *Crataegus berberifolia* T. & G. var. *Engelmanni* (Sarg.) Eggleston were collected April 28, 1929. Four soil samples were obtained.

Sample 1. This was obtained under cedar trees, about three-fourths up a slope of a dry, cherty and argillaceous limestone glade. *Dodecatheon Meadia* L., *Astragalus distortus* T. & G., *A. mexicanus* A. DC., and *Lithospermum canescens* (Michx.) Lehm. grew here. The soil was of a yellowish brown color, with a pH of. 8.00.

Sample 2. This sample was dug from dry soil on a slope in cedar woods, about halfway down a hill, where cherty to pure limestone rocks outcropped. The soil was deeper and less rocky here, and of a dark brown color. *Smilax ecirrhata* (Engelm.) Wats., *Polygonatum commutatum* (R. & S.) Dietr., *Botrychium virginianum* (L.) Sw., *Camassia esculenta* (Ker.) Robinson, and *Galium circaezans* Michx. were growing here. The sample was found to be subalkaline, its pH being 8.41.

Sample 3. This was collected on top of a cherty to pure limestone glade, in strong sun, in a large open area surrounded by cedars. The soil consisted almost solely of rock fragments. *Arenaria patula* Michx., *Scutellaria parvula* Michx., *Psoralea tenuiflora* Pursh, and *Petalostemum purpureum* (Vent.) Rydb. were the plant associates. The soil tested was circumneutral, its pH being 8.19.

Sample 4. This was from a similar locality to that of the previous sample, except that the glade was wider and cedars were

found only below and above the barren rock portion. Here were found *Oenothera missouriensis* Sims, *Viola pedata* L., *Sisyrinchium angustifolium* Mill., *Hypoxis hirsuta* (L.) Coville, *Brauneria angustifolia* (DC.) Heller, and *Coreopsis lanceolata* L. The soil was dry, exposed to the sun, and pure limestone rock predominated. It was found to have the same pH as that of sample 1, namely, pH 8.00.

ROUGH STONY LAND SOIL

The soil group classed under this head is derived from igneous rocks consisting of granites, rhyolites, trachytes, and diabase, the most abundant being a dense, hard porphyritic trachyte. The topography of this region is very rough.

One sample was collected from an area opposite Pilot Knob, in Iron County, on April 21, 1929.

Sample 1. This was from a dry sunny hillside opposite Pilot Knob, about a quarter of the distance up a 400-foot slope. It was taken from between rocks of porphyritic trachyte, surrounded by huge boulders. The trees consisted chiefly of second- and third-growth oak and hickory. The ground plants found here associated were *Tradescantia brevicaulis* Raf., *Vaccinium arboreum* Marsh., *Viola pedata* L., and *V. palmata* L. The soil was grayish brown in color, and its pH was 7.089.

CLARKSVILLE SOILS

These soils are mainly stony loams and are derived from the upper Cambrian (Ozarkian) beds of Gasconade cherty limestone, with a basal formation of Gunter sandstone. The areas of Clarksville soils are the most thoroughly dissected of any of the important soil areas of the Ozark dome. One sample was obtained through the kindness of Miss Marion Child, who dug it in Pulaski County, about twelve miles southwest of Dixon, on March 31, 1929.

The sample was obtained on top of a sun-exposed bluff which faced the Gasconade River. There were outcroppings of the Gunter sandstone, and the soil was a fine, sandy, cherty loam, brownish-red in color. Red cedars grew plentifully in the area. Other plants found here were *Verbena canadensis* (L.) Britton, *Lithospermum canescens* (Michx.) Lehm., and *Verbascum Thapsus* L. The soil was circumneutral, its pH being 7.819.

It will be seen from the foregoing account of the work that most of the soils ranged from minimacid to subalkaline, only five of the twenty-four soils tested showing any marked acidity.

No broad generalizations can be made from the limited range of the present piece of work, since the soils were obtained from comparatively few areas, and none was worked in detail. The effort in this investigation was to obtain a reconnaissance of the soil acidities of eastern Missouri, with a list of some characteristic plants on each soil type.

As stated by others, the fact that a given plant is found in soils of a certain degree of acidity or alkalinity does not necessarily indicate that the pH concentration is the all-important factor in determining where the plant grows; nor even that it acts directly upon the plant. It is the opinion of plant physiologists generally that the question of pH has been unduly emphasized as the dominant factor in plant distribution in relation to soil acidity. It appears more and more evident that the distribution of any given plant is the result of a number of factors; of these factors soil acidity or alkalinity and its relationship with hydrogen-ion concentration may be of significance or it may not.

Wherry ('20) has shown that plants grow in nature only when the hydrogen-ion concentration is within certain limits. Sometimes the range may be quite large, and at other times quite narrow. Arrhenius ('20) has studied the "Skärs" around Stockholm, Sweden, and he finds that among the factors influencing plant distribution hydrogen-ion concentration plays a very important rôle. Atkins ('22), in Ireland, is another to have studied the relation between plant distribution and soil acidity. Braun-Blanquet ('24), studying the vegetation of the Mediterranean, found that the hydrogen-ion concentration seems to be the factor in determining the distribution of the so-called calcicoles (lime-growers) rather than the lime. It is thus seen that several investigators in widely separated places have found that plants in nature are greatly influenced by the active acidity of the soil.

Wherever possible the results of the present work were compared with those of Wherry, and in most cases the results checked well. In a number of instances, however, it was found that whereas Wherry had placed a species in a definite class, the present

work indicated that this species is more or less indifferent and grows in a wide acid range. For instance, *Viola pedata* L. is almost always referred to as a subacid to minimacid soil plant, whereas the present work showed it has a range from pH 5.89 to pH 8.448, or in other words, from subacid to decidedly subalkaline. Time and again, *Viola pedata* L. was found on limestone substratum, a fact that would indicate alkalinity. In the case of this plant, which grows usually in dry, sunny, rocky or mossy places, the question appears to be one concerned with water content in the soil rather than of soil acidity.

Other examples of apparent differences are as follows: (1) *Botrychium virginianum* (L.) Sw. is classified as a subacid soil plant; the present work shows this species taking subalkaline conditions. (2) *Hypoxis hirsuta* (L.) Coville and *Lithospermum canescens* (Michx.) Lehm., usually classified as subacid soil plants, were found to take minimalkaline conditions.

There are other apparent instances, also. The present work would lead the writer to believe that there are many plants it would be erroneous to treat as of a definite soil type, for results show that usually these plants are indifferent towards soil pH and will accept quite a range of acidity and alkalinity. Such plants, it is felt, seem to be influenced greatly by water content of the soil or by a combination of other factors, in addition to that of soil acidity. In some cases, it seems unquestionably true that the distribution of certain plants is affected by the soil acidity; in some cases this soil acidity can be traced back to the water relationship in the soil, and in others it cannot. On the other hand, very often the factor of soil acidity does not seem to be the most important one to be considered. It would seem that a number of factors in certain combinations or ratios have much to do with affecting the distribution of a plant, rather than any single factor, such as that of soil acidity.

This work was carried on in the spring of 1929 in the Plant Physiological Laboratory of Washington University, under the kind supervision of Dr. E. S. Reynolds.

COMPARISONS BETWEEN WHERRY'S SOIL ACIDITY RESULTS AND THOSE OF THE PRESENT WORK

Plant	Soil type	Acidity found in present work	Acidity found by Wherry
Lycopodium lucidulum Michx.	Tilsit	pH 4.898 (mediacid)	
Castilleja coccinea (L.) Spreng.	Hagerstown	pH 5.806 (subacid)	
**Krigia amplexicaulis* Nutt.	Hagerstown	pH 5.806 (subacid)	
Pedicularis canadensis L.	Hagerstown	pH 5.806 (subacid)	Minimacid
**Heuchera hirsuticaulis* (Wheelock) Rydb.	Hagerstown	pH 5.806 (subacid)	Indifferent
Geranium maculatum L.	Hagerstown	pH 5.806 (subacid)	Indifferent
**Erigeron pulchellus* Michx.	Hagerstown	pH 5.806 (subacid)	Indifferent
Polystichum acrostichoides (Michx.) Schott	Hagerstown	pH 5.806 (subacid)	Indifferent
Ranunculus fascicularis Muhl.	Hagerstown	pH 5.806 (subacid)	Minimacid
Talinum teretifolium Pursh	Tilsit	pH 5.85 (subacid)	
Polytrichum commune L.	Tilsit	pH 5.85 (subacid)	
Krigia Dandelion (L.) Nutt.	Tilsit	pH 5.89 (subacid)	
**Tradescantia bracteata* Small	Tilsit	pH 5.89 (subacid)	
Baptisia bracteosa (Muhl.) Ell.	Hagerstown	pH 5.89 (subacid)	
**Viola pedata* L.	Hagerstown	pH 5.89 (subacid)	Subacid and Minimacid
**Monarda Bradburiana* Beck	Hagerstown	pH 5.89 (subacid)	
**Antennaria plantaginifolia* (L.) Richards.	Hagerstown	pH 5.89 (subacid)	Minimacid
**Polygonatum commutatum* (R. & S.) Richards.	Hagerstown	pH 5.89 (subacid)	
Dicentra canadensis (Goldie) Walp.	Tilsit	pH 6.58 (circum-neutral)	Circumneutral
Dicentra Cucullaria (L.) Bernh.	Tilsit	pH 6.58 (circum-neutral)	Circumneutral
Phlox divaricata L.	Hagerstown	pH 6.826 (circum-neutral)	Circumneutral
**Erigeron pulchellus* Michx.	Hagerstown	pH 6.826 (circum-neutral	Circumneutral
**Krigia amplexicaulis* Nutt.	Hagerstown	pH 6.826 (circum-neutral)	Circumneutral
**Tradescantia bracteata* Small	Hagerstown	pH 6.826 (circum-neutral)	Circumneutral
Cornus florida L.	Hagerstown	pH 6. 826(circum-neutral)	Minimacid
Rosa humilis Marsh.	Hagerstown	pH 7.081 (neutral)	
Vaccinium vacillans Kalm	Hagerstown	pH 7.081 (neutral)	Subacid
**Antennaria plantaginifolia* (L.) Richards.	Hagerstown	pH 7.081 (neutral)	Minimacid
Cunila origanoides (L.) Britton	Hagerstown	pH 7.081 (neutral)	
Rubus occidentalis L.	Hagerstown	pH 7.081 (neutral)	
Tradescantia brevicaulis Raf.	"Rough stony land"	pH 7.089 (neutral)	Subacid
Vaccinium arboreum Marsh.	"Rough stony land"	pH 7.089 (neutral)	
**Viola pedata* L.	"Rough stony land"	pH 7.089 (neutral)	Subacid or Minimacid
Viola palmata L.	"Rough stony land"	pH 7.089 (neutral)	
Orchis spectabilis L.	Tilsit	pH 7.2 (neutral	Circumneutral
Aplectrum hyemale (Muhl.) Torr.	Tilsit	pH 7.2 (neutral)	
**Corallorrhiza maculata* Raf.	Tilsit	pH 7.2 (neutral)	

Plant	Soil type	Acidity found in present work	Acidity found by Wherry
Acorus Calamus L.	Hagerstown	pH 7.285 (neutral)	
Mentha spicata L.	Hagerstown	pH 7.285 (neutral)	
Typha latifolia L.	Hagerstown	pH 7.285 (neutral)	
**Monarda Bradburiana* Beck	Hagerstown	pH 7.438 (circumneutral)	
Zizia aurea (L.) Koch	Hagerstown	pH 7.438 (circumneutral)	Circumneutral
Parthenium integrifolium L.	Hagerstown	pH 7.438 (circumneutral)	
**Brauneria angustifolia* (DC.) Heller	Hagerstown	pH 7.438 (circumneutral)	
Sullivantia renifolia Rosendahl	Tilsit	pH 7.65 (circumneutral)	Indifferent for *S. Sullivantii* (T. & G.) Britton
**Hydrangea arborescens* L.	Tilsit	pH 7.65 (circumneutral)	
Marchantia polymorpha L.	Tilsit	pH 7.65 (circumneutral)	
Hypoxis hirsuta (L.) Coville	Union	pH 8.00 (circumneutral)	Subacid
Sisyrinchium angustifolium Mill.	Union	pH 8.00 (circumneutral)	
Oenothera missouriensis Sims	Union	pH 8.00 (circumneutral)	
**Brauneria angustifolia* (DC.) Heller	Union	pH 8.00 (circumneutral)	
**Viola pedata* L.	Union	pH 8.00 (circumneutral)	Subacid or minimacid
Dodecatheon Meadia L.	Union	pH 8.00 (circumneutral)	Circumneutral
Astragalus distorta T. & G.	Union	pH 8.00 (circumneutral)	
Astragalus mexicanus A. DC.	Union	pH 8.00 (circumneutral)	
**Lithospermum canescens* (Michx.) Lehm.	Union	pH 8.00 (circumneutral)	Minimacid
Arenaria patula Michx.	Union	pH 8.19 (circumneutral)	
Scutellaria parvula Michx.	Union	pH 8.19 (circumneutral)	
**Psoralea tenuiflora* Pursh	Union	pH 8.19 (circumneutral)	
Petalostemum purpureum (Vent.) Rydb.	Union	pH 8.19 (circumneutral)	
Corallorrhiza maculata Raf.	Hagerstown	pH 8.227 (slightly subalkaline)	
Viola striata Ait.	Hagerstown	pH 8.227 (slightly subalkaline)	
**Botrychium virginianum* (L.) Sw.	Hagerstown	pH 8.227 (slightly subalkaline)	Subacid
Orchis spectabilis L.	Tilsit	pH 8.26 (slightly subalkaline)	Circumneutral
Smilacina racemosa (L.) Desf.	Tilsit	pH 8.26 (slightly subalkaline)	Indifferent
Panax quinquefolium L.	Tilsit	pH 8.26 (slightly subalkaline)	

Plant	Soil type	Acidity found in present work	Acidity found by Wherry
Mertensia virginica (L.) Link	Tilsit	pH 8.278 (slightly subalkaline)	Circumneutral
**Aquilegia canadensis* L.	Hagerstown	pH 8.363 (subalkaline)	Circumneutral
Cystopteris bulbifera L.	Hagerstown	pH 8.363 (subalkaline)	Circumneutral
Smilax ecirrhata (Engelm.) Wats.	Union	pH 8.41 (subalkaline)	
**Polygonatum commutatum* (R. & S.) Dietr.	Union	pH 8.41 (subalkaline)	
**Botrychium virginianum* (L.) Sw.	Union	pH 8.41 (subalkaline)	Subacid
Camassia esculenta (Ker.) Robinson	Union	pH 8.41 (subalkaline)	
Galium circaezans Michx.	Union	pH 8.41 (subalkaline)	
**Psoralea pedunculata* Pursh.	Hagerstown	pH 8.448 (subalkaline)	
Agave virginica L.	Hagerstown	pH 8.448	
**Viola pedata* L.	Hagerstown	pH 8.448	Subacid
Houstonia longifolia Gaertn.	Hagerstown	pH 8.448	
Oxalis violacea L.	Hagerstown	pH 8.448	Indifferent
Cheilanthes Feei Moore	Hagerstown	pH 8.482	Circumneutral to subacid
**Aquilegia canadensis* L.	Hagerstown	pH 8.482	Circumneutral
**Hydrangea arborescens* L.	Hagerstown	pH 8.482	
**Heuchera hirsuticaulis* (Wheelock) Rydb.	Hagerstown	pH 8.482	Indifferent

* Denotes wide range of pH.

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STUDIES ON THE PHYSIOLOGY OF PLANT DISEASE

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I. General Considerations

The study of the physiology of plant disease has been rather incidental to the work of the pathologist although of basic significance both theoretically and practically. Considerable material concerning the subject is scattered throughout botanical writings, and in recent articles references to various portions of it are made indiscriminately. Many contradictions, real or apparent, are accepted because no discriminating summarization is available, and as a result the objectives of investigation are often vague. A very urgent need exists for a thorough survey of the field, together with a careful consideration of the bearings of recent investigations in the contributory lines of work.

Because the subject is extremely complex it is especially necessary to separate clearly from one another the various questions involved. In many diseases parasitism does not exist, and a careful study of the physiology of such abnormal conditions in contrast with the normal physiology of the plant is especially important. In the study of parasitic diseases the parasite, the host, and the host-parasite complex must be studied separately in relation to the environment. This means, among other things, a better knowledge of the nutritional habits of each disease-producing fungus, although it must be recognized that the substances which a fungus may use as food are often much more numerous than those with which it can come in contact in nature. Here again the scattered information should be brought together and carefully analyzed as a basis for further study.

Such suggestions as we have concerning resistance, at the present time, indicate that there are several types which form a more or less closely graded series. We should scarcely look for any generalization to explain so complex a set of phenomena, nor should we deceive ourselves in the use of such a broad term as "resistance," into expecting to discover some such generalization. Perhaps one of the most frequent mistakes among scientists is to

discover some new law or generalization and then attempt to apply it to many different series of facts. Because natural selection is an important fact of nature we should not attempt to ascribe all evolution to its action. Similarly because we recognize that resistance may be due, in certain cases, to toxic materials, we should not discard the evidence of probable morphological causes in other cases, nor should we fail to recognize that several independent causes may operate conjointly.

At least two rather different classes of disease, as regards resistance, should be differentiated on the basis of the type of parasitism. Certain diseases, such as the rusts, smuts, and powdery mildews, as well as others less well known, are caused by fungi which either cannot grow at all in artificial culture media such as we usually prepare, or if so it is only in a limited portion of their life history and they will not complete their life cycle under such conditions. These are the diseases caused by obligate parasites. Other diseases are caused by fungi which may more or less readily be cultivated as saprophytes in culture media throughout a large portion, if not all, of their vegetative life and often their reproductive phases. Such fungi, which usually are saprophytic but may attain a parasitic life, or on the other hand usually are found as parasites but may be cultivated as saprophytes, are referred to as facultative organisms. We should expect to find intermediate conditions between these two general types of disease, but for clarity of vision it is often well to differentiate sharply the extremes of even a continuous, progressive series.

Resistance in diseases due to obligate parasites may be quite different from that in diseases due to the semi-parasitic, semi-saprophytic fungi. The obligate parasites appear to have been associated with their hosts for long periods of time, resulting in their having become highly specialized to them. This suggests in turn a probability that they are rather stable in their needs and their life habits. If the observations of Stakman, Parker and Piemeisel ('18) concerning the black stem rust of wheat are to be taken as typical of the obligate parasites, some evidences are before us that there is little or no mutation of these fungi taking place now. On the other hand, Leonian's studies ('29), together with others, indicate that the opposite is true of the wilt fungi and

other partial parasites. Their specialization also is less marked in general. Resistance to such omnivorous feeders may well be quite different from immunity or resistance to the highly specialized obligate parasites. Since all organisms are somewhat plastic in their environment the similar effect of environment upon the parasitic relationship in these two classes of disease need not be considered as an argument against the main thought just expressed. The possibility or even probability that in at least some diseases of the facultative-parasitic group mutations of the pathogene may occur disturbs our feeling of security in obtaining resistant varieties by breeding. This should stimulate us to study the causes of resistance, thus having at hand further knowledge to aid us in the fight against possibly saltating parasitic qualities.

We recognize that in some types of disease avoidance the causative organism is excluded so that it does not enter the so-called resistant variety. Thus McLean ('21) reports that in the Mandarin orange, which does not take the citrus canker disease, the stomata are so constructed that they prevent entrance of water and of the bacteria. When the epidermis is injured and the bacteria enter, the supposedly immune variety becomes diseased. Such mechanical conditions seem to be illustrated again in the maturing tomato skin (Rosenbaum and Sando, '20). The fruit develops a thicker cuticle which resists more and more, either the puncturing of a needle or that of the germ-tube of *Macrosporium tomato*. Other kinds of disease avoidance are accomplished through early maturing of varieties, or the absence of insect carriers. Hardly either of these latter types should be called true resistance.

A considerable number of fairly well-authenticated cases are now known in which susceptibility to disease is in direct proportion to the general degree of loss of vegetative vigor. Thus Jones ('99, '05) early reported that the late blight fungus attacks potatoes at the so-called "critical" period of the life of the host, when the greatest drain occurs upon the life processes. The resistance of "Little Jess" wheat to rust is reported by Weston ('27) as breaking down when this variety is attacked by the bunt fungus; and other varieties of wheat likewise become more heavily rusted under attack from bunt. Is resistance more common

among the wild than among the cultivated plants, as has been suggested by some, or the reverse as others have believed? Assuming a struggle for existence, we would expect the wild plants to show the greater resistance, since cultivated ones, while perhaps not lower in vitality, as some have supposed, have been selected by primitive man for other reasons than disease resistance and the latter quality has been more or less lost in the process. Natural selection has resulted in disease resistance among wild plants where these have come in contact with the pathogene. Rapidly growing, vigorous plants frequently are more subject to fungous attack than those of the same species which are slow growing. If resistance is at times due to the presence of some specific, toxic substance it is entirely possible that the reason for the ease of attack on rapidly growing specimens is because this characteristic toxin has not accumulated as rapidly as it can accumulate in slower growing individuals. Metabolic processes resulting in characteristic biochemical products do not always proceed at the same pace as the growth processes which result in enlargement. It is not probable, however, that plants in a condition of reduced vitality are often less subject to invasion, although statements with that implication appear from time to time. These are usually based upon observations, often among rust diseases, which indicate that the hosts which are not carrying on rapid photosynthesis are less vigorously attacked by the pathogene. Doubtless the greater abundance of food in those hosts with active photosynthesis would account for the greater growth of the parasite. Practical criteria for recognition of general physiological, potential vitality or vigor, as contrasted with rapid growth and active photosynthesis, might eliminate these cases from the field of resistance studies.

The usual inability of a pathogene to infect many different kinds of plants need not be due to any particular "resistance" on the part of the unattacked plants, but simply to a complete non-relationship between them and the fungus. No doubt a careful survey would show that many of the fungi which are semiparasitic could be induced to grow upon numerous plants not naturally their hosts and cause disease in these new hosts. Thus new relationships would become established. Massee ('25) pro-

duced parasitic life habits in pure saprophytes by first cultivating them on leaves of plants injected with sugar for several generations and then finally upon the same kinds of leaves without the sugar injection. He also cites the case of the fungus *Dendryphium* which is a pure saprophyte, but which under exceptional conditions in the greenhouse was able to assume a parasitic life upon the cucumber and cause disease injury. Kunkel ('26), it would seem, has established a condition somewhat similar to induced parasitism in his transference of Aster yellows to many plants not naturally subject to the disease, and Young ('26) has made a similar study for several facultative parasites.

Parasitism is a struggle between two organisms, which may be over-balanced by the environmental complex in one direction and result in death or serious injury to the host. It may similarly be over-balanced in the other direction either by external environment or by internal conditions and result in various degrees of resistance; or the balance may be essentially even, in which case such organisms are rather well adjusted to one another and little injury results. Lichens may be suggested as examples of the balanced condition. There are all grades between the two extremes. How then, since environment plays such an important rôle, may we speak of some specific quality, character, or substance as "the cause" of resistance? We are all well aware that in animal and plant parasitic diseases certain causative organisms must be present before the disease occurs. We also know that in most cases certain environmental and internal conditions must be present in addition. Nevertheless we call the specific pathogene "the cause" of the disease. In quite a similar manner it may be possible to pick out of the complex of conditioning phenomena of resistance to a given disease, one factor without which no combination of external environmental factors could cause resistance. This would be called similarly "the cause" of resistance. This may be thought of as the inherent, hereditary quality analogous to "height" as a genetic character, which is subject to such wide fluctuations that an individual of one height-class may be somewhat taller or shorter than some individuals of a neighboring height-class. The discovery of this hereditary cause of resistance in any specific case would not end the problem, since the relative

importance of the various external factors should be clearly estimated. This requires a physiological study of the effects of the disease upon the host as well as what we may call the predisposing causes of the disease. Jones ('26) has recently well outlined the necessary coöperative spirit by which rapid production of resistant varieties may take place, and such coöperative spirit would greatly aid in the general study of the physiology of plant disease.

A considerable portion of the observations in the past upon resistant varieties is invalidated by our present knowledge that there are biological variants of the causal organisms; and that different environmental conditions may quite over-balance the hereditary factor. It will be especially necessary as an early step in the analysis of the general problem to determine anew under what definite conditions each variety is resistant. Then the biochemical and physiological circumstances of resistance may more readily be determined. Much of the contradictory evidence concerning the cause of resistance may be cleared away by studying carefully individual pure lines as regards host resistance in relation to the various strains of the parasite and also in relation to the environmental factors affecting both host and parasite and the host-parasite complex.

Where then should we concentrate our renewed attack upon this general problem? It appears rather probable that the obligate parasites are not mutating rapidly and therefore the securing of resistant varieties is somewhat of a permanent advance. The opposite situation is more probable with the less highly specialized parasites, so it would appear most desirable to begin with the diseases caused by this group of organisms. An added incentive in the same direction arises from the hope that such less-specialized diseases may yield their secrets somewhat more readily and thus form a basis for the more difficult problems of the obligate parasitic diseases. The greater ease of manipulating the infective material also favors this approach.

The tendency of an invading organism is apparently toward chemical antagonism, killing of the host, and the use of the nonliving organic remains. The facultative organisms often accomplish this, while the obligate parasites fall short. Why? The

flecking of the highly resistant wheat plants by the invading rust and the death of the invading organism indicate a toxic host action rather than a lack of food for the fungus. A similar conclusion appears to be true as regards the potato strains resistant to the wart disease. Are parasitism and resistance reciprocals? There has been a rather strong feeling that if it could be known why a fungus invades the host, we would be well toward the solution of resistance. Thus Massee ('25) believed that the presence of attractive substances in the host causes chemotropic stimulation of the fungus and that this accounts for parasitism. The lack of these attractive chemical substances would cause resistance. However, may not the parasitism of the rust on wheat be due to the presence of transitory food factors, while the resistance of certain varieties be due to the presence of toxic material? Massee failed to educate certain fungi to become parasites, yet succeeded with others, indicating possible materials in plants which are toxic to some fungi and not to others. Obligate parasites may possibly require specific, labile substances in the host to which they have become specifically bound. This could hardly be true in the alternative type of disease in which the pathogene usually grows exceedingly well on a great variety of media. In this latter type, therefore, if resistance is due to a biochemical factor it is more likely concerned with the presence of toxic materials than with the absence of food factors.

The wide distribution of certain classes of specialized products in the plant kingdom, many of which are toxic to fungi, suggests strongly that resistance may often be associated with the relative abundance of one or more such substances. This would accord well with the fact that most kinds of resistance are relative in amount and vary with different varieties of the same host. Several workers have clearly demonstrated the toxic effects of tannins and of certain organic acids, alkaloids, and glucosides upon fungi. Walker ('29) and his co-workers (Link, Dickson and Walker, '29; Angell, Walker and Link, '30) seem to have definitely demonstrated that the presence of protocatechuic acid in the outer scales of the red and yellow onions is the cause of resistance of these varieties to the onion smudge disease. It is important then to study a definite fungus in relation to the special

chemical products of the hosts upon which it grows naturally or by artificial inoculation. It is clear that we must know much more of the specific compounds produced by plants rather than be satisfied with routine analyses for carbohydrates, total nitrogen, and the like. That resistance is in certain cases associated with chemical materials is indicated in many ways. It is reported that injection of malic, tartaric, and citric acids into the roots of apple trees made them immune to certain diseases. Apple and peach stocks were made resistant to *Oidium farinosum* and *Exoascus deformans* by the grafting in of resistant wild scions. The toxic action of certain plant juices to fungi of parasitic habit as described later on indicates the same conclusion.

It is possible that in certain cases the resistant quality is effective against several diseases of a similar type, although caused by fungi of diverse relationships. Vavilov ('14) cites *Triticum monococcum* as being immune to brown and yellow rusts and stinking smut, and resistant to the powdery mildew. *Triticum durum* and *T. polonicum* likewise are resistant to both brown and yellow rust and mildew. Conversely, diseases of different types may show no similarity in their lists of resistant varieties. Thus Italian clovers are susceptible to anthracnose and resistant to mildew, while some American strains are resistant to anthracnose and very susceptible to mildew (Monteith, '24).

Data on resistant varieties need very careful analysis, since even in the same disease and on the same host there may be two or more interacting causes of resistance, i. e., some physiologic, some morphologic, and some environmental, or combinations of these. Evidence of this is seen in the lack of complete agreement in the results from greenhouse and field experiments upon resistant varieties. The observation that black stem rust of wheat grows only in the chlorenchymatous collenchyma tissue and not in the sclerenchyma (Hursh, '24), together with many other observations on probable chemical influences, indicates the complex nature of certain kinds of resistance. Genetical studies also show that while some resistance apparently is due to a simple factor, in other cases there are multiple factors concerned.

From some experiments reported by Vavilov ('14) it appears probable that within the same species the cause of resistance may

vary with the different varieties of host in relation to the same disease. A Persian wheat was found immune to the powdery mildew, *Erysiphe graminis*. Other varieties of wheat are also at least highly resistant to the same disease, yet crosses of Persian on susceptible varieties gave immune F_1 plants, while other resistant varieties crossed on susceptible ones gave susceptible plants in the hybrid generation.

From the examples referred to in this discussion it appears that when viewed as a whole the subject of disease resistance is exceedingly complicated. However, a careful analysis of the various factors involved in any given disease followed by experiments planned to test these, one at a time, will make it possible to approach closely a solution of the problem. In a similar manner the various fundamental physiological bases of pathological phenomena can be attacked and definite progress made. These advances can hardly be made as a mere incident to routine pathological studies, but must be a major project of experimentation.

We may summarize, as follows, the preceding discussion:

1. A careful collation of past observations is needed, taking care that they shall be checked by recent results and by the results in contributing fields of investigation.

2. There should be a clearer recognition of possible types of resistance as based upon the various types of parasitism, though the causes of these two phenomena may not be related.

3. The environmental effects upon disease should be analyzed as far as possible into the effects upon the host, upon the parasite, and upon the host-parasite complex.

4. An open mind should be kept toward the probability that there is no general explanation for parasitism or for resistance, but rather a specific one for each disease which may involve several factors or only one.

5. Close coöperation among biochemists, physiologists, and pathologists is most urgent.

6. Special attention must be given to the individual, characteristic chemical products of each plant studied, as related to their toxicity to the disease-producing organisms which occur on that host.

7. The study of the fundamental problems in pathology should be treated as a special field of inquiry worthy of attention in its own right and not simply as an adjunct to the study of methods of combating disease.

Considerations such as have just been discussed have led to a project for the study of some of the biochemical relationships of certain disease-producing fungi and their host plants. Some investigations in this field are reported in the following sections.

II. THE GLUCOSIDE CONTENT OF FLAX

During a study (Reynolds, '26) of the nutritional relations of *Fusarium lini*, which is the causal agent of the wilt disease of flax, it was found that extracts of flax are poisonous to this fungus. This toxic quality was manifest in Fermi's medium which is a relatively poor one for the growth of *Fusarium lini*. It also appeared that an extract from a strain of flax which resisted the parasitic attack of this fungus was more toxic than one from a non-resistant flax. It was suggested that the hydrocyanic glucoside of flax might be the cause of this toxic quality since *Fusarium lini* is very sensitive to the presence of hydrocyanic acid in culture media (Reynolds, '24). It seemed desirable therefore to determine as accurately as possible the quantitative occurrence of this glucoside and to study further the toxic nature of flax.

Numerous more or less complicated methods of estimating the quantity of hydrocyanic acid produced by plant materials have been described and tested. None of these seemed satisfactory, since the purposes of the estimations and the nature of the plant materials were different from those of the proposed study. In the present investigation it was desired to make use of the flax material, after the estimation for fungous culture studies, with as little change in its characteristics as possible, and hence it was decided to use the Roe aeration method of extraction (Roe, '23, '24) modified as might become necessary.

The best procedure followed was to grind the green or dry flax material, add a definite proportion of water, and let it stand over night. This allows time for the specific enzyme, linase (Armstrong and Eyre, '12; Eyre, '12), which is in the flax, to hydrolyze the glucoside into HCN, glucose, and acetone. In order to get a

thorough mixing of the plant material and a complete aeration during the process of extraction, a gas-tight, motor-driven stirrer modified from one described by Hiers ('26) was used and fitted into the flask containing the flax mash, as soon as possible after the grinding. A preliminary freezing of the green flax and grinding in the refrigeration room at a temperature below the freezing point of water further conserved the HCN by preventing enzyme action until the water had been added and the stirrer put in place. The aeration was accomplished by a slow evacuation of the stirrer-flask, thus pulling a stream of air through an inlet tube opening well below the surface of the mash, and bubbling it out into a train of three gas washing bottles made up with Folin's ammonia tubes and containing a 5 per cent potassium hydroxide solution (fig. 1). The hydrocyanic acid, being very volatile, readily passed with the air into the hydroxide where it was changed into a weak potassium cyanide. Only rarely was any cyanide discovered in the third bottle of the train. By titrating this alkaline-potassium cyanide solution with a standard one hundredth molar silver nitrate in a manner somewhat similar to that recently reported by Bishop ('27), it was possible to get a very accurate determination of the amount of HCN caught in the hydroxide. For each experiment the percentage dry weight of each strain of flax was determined and the HCN content calculated in terms of the dry weight of the material used in the estimation. Numerous precautions and tests, unnecessary to detail here, were used in order to insure comparable results in the same experiment. While not all of the glucoside present in the flax could be determined in the HCN thus evolved, estimations run directly in comparison with one another under identical conditions and upon identical materials (Exps. 69 & 70) showed that such a method gave excellent checks.

Seven different strains of flax supplied by Professor H. L. Bolley of the North Dakota Agricultural Experiment Station and selected by him for different degrees of resistance to the flax wilt disease were used. Professor Bolley's designation of the relative resistance of these seven strains of flax is as follows:

1—NDR 114—Very resistant.

2—NDR 119 (Buda)—Very resistant.

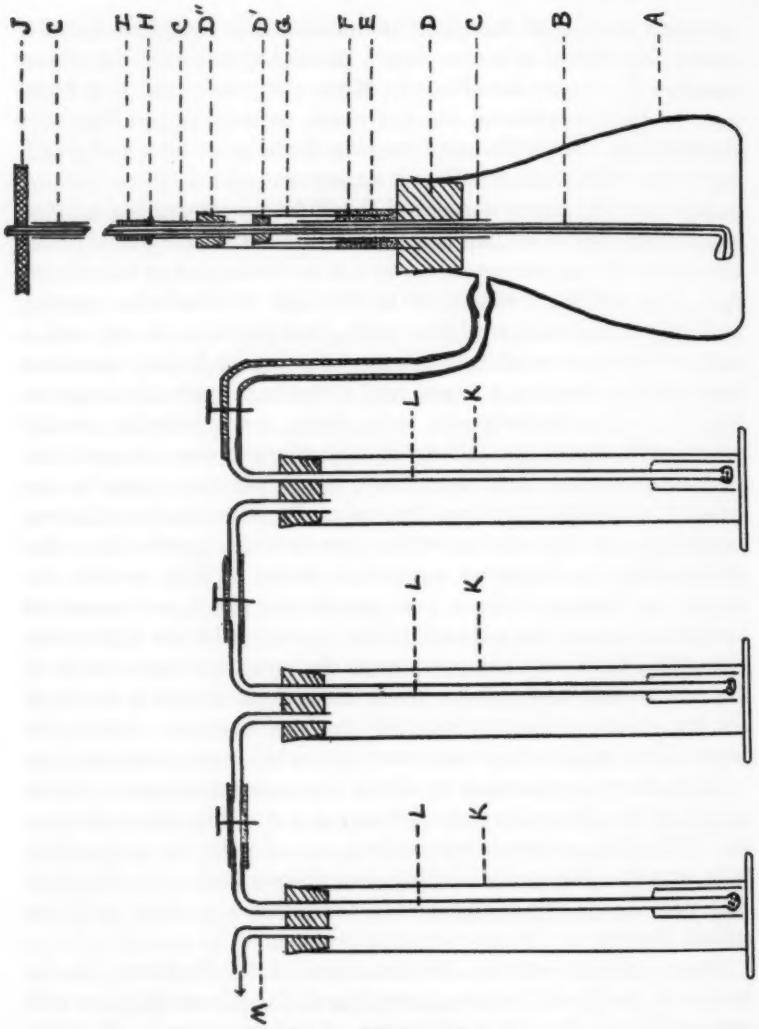

Fig. 1. Key to symbols.

A—One liter, or 500-cc., wide-mouth, side-neck, pyrex flask, with ground flax material in water.
B—Heavy-walled, glass tubing, 7 mm. o. d., bent into a paddle and with the bore left open at both ends. Air enters at the top.
C—Bearing tubes of 8 mm. i. d. with heavy grease as lubricant.
D, D′, D″—Rubber stoppers, # 7 or # 8, # 0, and # 3 or # 4, respectively.
E—Tube 23 mm. by 140 mm. Lower end buried in D.
F—Mercury seal.
G—Tube 16 mm. by 80 mm.
H—Loose metal washer.
I—Heavy-walled rubber tubing.
J—Wooden or cork pulley.
K—Cylinders with 5 per cent KOH solution.
L—Folin ammonia absorption tubes.
M—Attachment for suction.

3—L-79—Good resistance.
4—Cross—Breaking up—Fair wilt resistance.
5—Cross—Fairly good resistance.
6—N.D. 155—Tall fiber—large seed. No resistance on our sick plots.
7—N.D. 1215—No resistance under our conditions.

Different ages of flax and different environmental conditions of growth were also tested in an effort to determine their effects upon the amount of glucoside produced. The HCN in whole plants and in roots and shoots separated was also determined. In all, 66 separate estimations were carried out, besides a considerable number of preliminary and partial tests. The numerical data for all these determinations are given in table I. The percentage of HCN is calculated on the basis of the dry weight of the flax material. From .2 to .3 per cent HCN, corresponding to about 2 to 3 per cent glucoside, is a very high content, while about 1 per cent glucoside is a normally high content. Less than .1 per cent glucoside is a very low quantity for this series of experiments, except in fully matured flax which has almost none.

The following table gives a series of analyses made in England by Dunstan, Henry and Auld ('06), which illustrates the usual rise and fall of glucoside content in the flax plant from seed to seed. These are minimum quantities, since the method of analysis does not fully conserve the hydrocyanic acid present in the plants, and there are evidences from the experiments reported in the present paper that the glucoside content of some of the strains tested is considerably higher than shown in table II.

Since the experiments reported here were often designed to test the effect of age, environmental conditions, and various methods of determining the cyanogen content, it is evident that in most cases the numerical data from different experiments are not directly comparable. Two or more strains of flax, listed under the same experiment number and of the same age, were generally handled in a similar fashion and the data are usually comparable. It appears from a consideration of all this data that the quantity of HCN which can be evolved from the plant material is strongly influenced by the environmental conditions which have surrounded the growing plants and that a change of such conditions

TABLE I

SUMMARY OF HCN DETERMINATIONS

Exp. No.	Flax No.	Height (inches)	Age	Per cent HCN in flax strain Number								Notes
				1	2	3	4	5	6	7	11	
29	1		6½ wks.	.18								
31	7		7 wks.							.12		Shoots
										.07		Roots
33	4	5½–6	8 wks.				.12					Whole plants
34	2	7½–8½	8 wks.		.11							Whole plants
37	6	5½–6	8 wks.						.097			Whole plants
40	1	2½	5½ wks.	.12								High dry weight. Whole
42	6	4	6 wks.						.066			Whole plants
42	5	5½–6	6 wks.					.08				Whole plants
42	3	3½–4½	6 wks.			.075						Whole plants
42	2	4½–5	6 wks.		.085							Whole plants
43	2	3–3½	2 wks.		.083							Constant-light room
43	6	3–4	2 wks.						.11			Constant-light room
57	3	10–11	11 wks.			.045						
		7½–8				.078						From same flat
60	1	10–11	11 wks.	.20								Tops, 2–4 inches
61	1	Seedlings	5 days	.28								Stems. In dark
				.15								Roots. In dark
62	2	3–3½ (2–6½)	15 days		.054							Constant light. Poorly watered. Irregular height
62	6	4(2–7)	15 days						.046			Constant light. Poorly watered. Irregular height
64	6	10–14	8 wks.						.086			Low-temp. house, 52° C. night
64	6	7–11	8 wks.						.094			High-temp. house, 78° C.
66	2	10–14	8 wks.		.061							Low-temp. house
66	2	7–11	8 wks.		.069							High-temp. house
68	1	8½–10	6½ wks.	.031								Low-temp. house
68	2	8–9½	6½ wks.		.085							Low-temp. house
69	1	9½–12	7 wks.	.022								
				.020								Checks
70	7	4–4½	3 wks.							.127		
										.131		Checks
73	3	4–5½	3 wks.			.059						
74	2	6–9	16 days		.087							Constant light
74	6	7½–9½	16 days						.19			Plus 2 days ordinary
75	2	Seedlings	5 days		.35							Stems by aeration
					.062							Roots by aeration

TABLE I (*continued*)

Exp. No.	Flax No.	Height (inches)	Age	Per cent HCN in flax strain Number								Notes
				1	2	3	4	5	6	7	11	
76	2	Seedlings	5 days		.31							Stems } Juice filtered into KOH
					.051							Roots }
77	4	3½–4½	3 wks.				.11					59° C. at night
	6	3½–4½							.089			
79	2	3–4	16 days		.19							
82	2	7–8	30 days		.053							
	6	7½							.06			
83	1	1½–2	3 wks.	.08								Aeration faulty
83	4	1½	3 wks.				.13					
85	2	4–5	19 days		.18							
	5	4½–5½						.061				
87	1	3–4	30 days	.034								Slow-growing in cold-frame
	1	20–22	7–8 wks.	Trace								
90	4	3–5	5 wks.				.073					
91	1	2½–3	3 wks.	.015								
	6	2½–3							.011			In cold-frame
99	1	4½–5	4 wks.	.066								
	6	3–4	4 wks.						.056			In cold-frame
100	1	6–8	6 wks.	.019								In cold-frame
104	4	7½–9	6 wks.				.029					
108	2a	5–6	5 wks.		.063							Healthy
		3–4			.0083							Yellow
109	6	4½–6	5 wks.						.078			
112	1	4½–6½	7 wks.	.017								
	3	5–6				.009						
113	1	5–7	7 wks.	.006								Duplication of No. 112 but 2 days later
	3	6–7	& 2 days			.01						
114	2a	6½–11	7 wks.		.01							
	6	6½–8							.012			
125	2a	Seedlings			.094							Possible loss during storage
	7a	Seedlings								.027		
128	11	2–4	2 wks.								.243	
136	1a	3–3½	12 days	.11								
145	11	5–10	5–6 wks.								.20	

Note:—Nos. 1a and 2a a different year's production of seed; No. 11 a highly resistant selection from No. 1.

TABLE II

THE GENERAL COURSE OF HCN CONTENT OF FLAX THROUGHOUT THE LIFE OF THE PLANT

Height of flax plant in inches	Per cent HCN found	Per cent glucoside calculated
Seed	0.008	0.07
1–1.5	.15	1.4
2–3	.17	1.5
3–4	.15	1.4
4–5	.13	1.2
5–6	.10	0.9
6–7	.10	0.9
8–9	.08	0.7
12–15	.07	0.6
15–18	.03	0.3
18	0.009	0.08
18	None	None

for even a day or so may influence this quantitative characteristic immediately (Exps. 112 and 113). Willaman and West ('16) noted some climatic effect on the production of HCN by sorghum, although they were inclined to believe that varietal constitution caused greater modifications than did climatic differences. Pinckney ('24), however, was convinced that HCN was increased in direct proportion to the increase of nitrate fertilizer on a low nitrate field, and that yellowish sorghum produced little if any HCN when the green plants contained a good supply.

While in general young flax runs high in hydrocyanic glucoside and old flax low, yet it appears that neither age nor height as such are proper indicators of the probable HCN production. The condition which seems to influence this quantitative factor is the number of actively functioning cells. Thus plants of the same age, but of different physiologic vigor, may develop nearly eight times as much HCN in the normal healthy specimens as in yellow retarded ones (Exp. 108). Experiments 43 and 62, although originally intended to be duplicates, carried on in the constant-light room, do not show the same quantities of HCN. In the latter experiment, due to the crowded condition of the room, the flax flats were irregularly and inadequately watered. The resulting plants, although of equal age, were very unequal in size and vigor of growth. The amounts of HCN in both strains were noticeably less than in Experiment 43 in which the growth was

uniform and normal. In the examples cited the irregular watering of the flats seemed to be the factor which largely determined the relative vigor of the plants. The upper two to four inches of flax eleven weeks old, which was in vigorous physiologic condition, gave a high quantity of HCN hardly equalled even by very young flax (Exp. 60). Thus it would appear that the reason the percentage of HCN in flax shows a steady decrease from a maximum at 2–4 inches high is because a continually decreasing proportion of the plant is composed of actively functioning cells.

Temperature variations of medium range did not seem to modify the glucoside content (Exp. 64 and 66). The use of the constant-light room did not seem to cause any special change in the quantity either, although the life cycle was greatly shortened so that seed was matured in about half the usual time. The shoots of flax seedlings may contain five times as much glucoside as the roots (Exps. 75 and 76), while in older plants there is a smaller difference (Exp. 31), due mainly perhaps to the drop in the percentage in the shoot. In the older plants it is very difficult to get the smaller and hence physiologically more active roots for a quantitative determination, so that it is not known at present how much glucoside is in the whole root system. It is interesting to note that while fully mature flax seed contains very little, if any, glucoside which may be converted into HCN, five-day-old seedlings, which had developed in the dark, had the highest percentage of the acid which was found in this series of experiments (Exps. 61, 75, and 76).

The flax strains numbered 1, 2, 3 and 4 are, in the main, strongly resistant to the wilt disease, while No. 5 is intermediate and Nos. 6 and 7 are only slightly, if at all, resistant, as determined through field tests by Professor Bolley. Although in many of the analyses the more resistant varieties ran high in glucoside content and the less resistant ones low (table III) not enough data were gathered to prove definitely a correlation (Exps. 42, 77, 85, etc.). It may be noted from table III that the 2–6 pair, which in four out of seven tests showed a greater amount of HCN in the susceptible flax than in the resistant, accounts for four out of the nine cases in which this same relationship is seen. Under most conditions the No. 6 flax seems to produce more HCN than No. 2.

It may be that it has more glucoside or that under certain circumstances it produces a larger amount of linase, or a more active enzyme. Strain 4 consistently developed a high quantity of HCN for the conditions under which it was growing and constitutes the other apparent exception to a correlation between strong resistance and high glucoside content. However, Professor Bolley's characterization of its degree of resistance indicates that it is rather likely to be exceptional.

TABLE III

PAIRS OF FLAX VARIETIES COMPARED AS TO RELATIVE QUANTITIES OF HCN. R = MORE RESISTANT MEMBER OF PAIR; S = MORE SUSCEPTIBLE

Exp. No.	Pairs of varieties	R more HCN than S	S more HCN than R
42	5–6	*	
	2–3	*	
	2–6	*	
	3–6	*	
	3–5		*
	2–5	*	
43	2–6		*
62	2–6	*	
68	1–2		*
74	2–6		*
77	4–6	*	
82	2–6		*
83	1–4		*
85	2–5	*	
91	1–6	*	
99	1–6	*	
100, 104	1–4		*
112	1–3	*	
113	1–3		*
114	2–6		*
125	2–6	*	

It is evident from Broadfoot's work ('26) that each of the different strains of flax is resistant to the different strains of *Fusarium lini* in different degrees. Because of these considerations and of the variety of conditions surrounding the plants and the ease with which the glucoside content may change we must conclude that the lack of an evident, exact correlation does not disprove a possible causal relationship between resistance and glucoside production. Many more determinations of the glucoside and of the changes in amounts of linase in flax must be made before a clear

picture of the distribution and significance of HCN can be drawn. Such a study is planned for the near future.

While it had been determined formerly (Reynolds, '24) that potassium cyanide inhibits the growth of *Fusarium lini* on agar plates at a concentration of about .03M it was thought best to test the effect of cyanide in liquid cultures. The following experiment (No. 15) was made: A solution of hydrocyanic acid was prepared so that 5.2 cc. in a 50-cc. culture would give a .03M concentration of HCN. The standard culture medium described in the third section of the paper was used and a series of cultures made as indicated in table IV. Some of these were inoculated with No. 2 *Fusarium lini* and some with No. 7 *Fusarium lini*. The former was one of Broadfoot's ('26) most virulent flax parasites and the latter one of the least parasitic of his group. The cultures in the Florence flasks (F) were sealed with paraffin to

TABLE IV

EFFECT OF CYANIDE IN LIQUID CULTURES

F. lini culture	Amount of HCN sol. (cc.)	Style of flask	Average growth in milligrams of dry weight at		
			15° C.	20° C.	27° C.
# 2	0.2	F	*3—(4)	3—188.8	3—122.0
# 7	0.2	F	3—229.7	3—347.6	3—233.9
# 2	1.0	F	3—(6)	3—143.3	3— 90.0
# 7	1.0	F	3—(1)	3—188.7	3—(2)
# 2	5.2	F	2—none	2—none	3—none
# 7	5.2	F	1—none	3—none	3—none
# 2	1.0	E	2—(5)	2— 48.0	2— 75.1
# 7	1.0	E	1—Trace	2—222.3	2—300.7
# 2	5.20	E	2—none	2—(7)	2— 36.6
# 7	5.20	E	2—none	2—(7)	2—(3)
# 2	None	E		3—222.9	
# 2	None	F		3—266.1	2— 89.9
# 7	None	F	2—343.4	2—260.2	
# 7	None	E	3—220.7		

* The number preceding the dash indicates the number of cultures in the set. A number in parentheses refers to the following notes.

Notes:
(1) Two with possibly a trace of growth, and third no growth.
(2) One with undetermined amount of growth; one, trace; one, none.
(3) One culture, 31.4 mg.; one, trace.
(4) One culture, 33.9 mg.; one, 7 mg.; one with undetermined amount.
(5) One culture, 30.3 mg.; one, trace around inoculum.
(6) One culture, 21.4 mg.; two, undetermined amounts.
(7) One culture, trace; one, none.

prevent, as far as possible, the escape of HCN. The Erlenmeyer flasks (E) were closed only with cotton plugs.

It will be seen from the results tabulated that the greatest concentration of HCN is practically always completely inhibitive except at the higher temperatures in the non-sealed flasks. The irregular results here indicate that enough of the HCN was lost by diffusion into the air to reduce slightly the toxic quality. This is indicated somewhat also by the observation that nearly all of the HCN flasks in the 27° C. incubator which showed growth were on the lower shelf nearest the heating unit. It appears that the fungus was less resistant to the toxic effect of the HCN at 15° C. than at 20° C., as one would expect from the fact that the former temperature is not as favorable for this species as the latter. The irregular numerical results, especially of the higher concentrations, are characteristic of cultures which are near the inhibitive, toxic concentration of poisons. The No. 2 strain of *F. lini* is a less vigorous grower in this standard medium than the No. 7 strain, but it would seem that there is little difference between the two under the action of the HCN in culture.

The results of the analyses given in table 1 show that in the older flax roots the proportion of HCN to dry weight is much lower than in the shoot. However, they also indicate that in young roots, as found in seedlings, the percentage of HCN may rise to a considerable amount. It is stated by Armstrong and Armstrong ('10) that the entrance of certain substances into a plant which has a cyanogenetic glucoside causes a "cumulative" change, so that a very small portion of the substance produces a large relative production of HCN. If this is true it is entirely conceivable that the entrance of a fungus might likewise stimulate a concentration of the glucoside at the point of attack. Hence the normal concentration of HCN, as determined by analyses such as reported here, does not necessarily have to indicate a toxic concentration in order to account for resistance.

While the various experimental evidences and the considerations just discussed do not give positive proof either for or against the causal relationship between the presence of the cyanophoric glucoside in flax and a certain varietal resistance to the wilt disease, yet it would seem that at least a part of the resistant

quality may be attributed to this chemical condition. In view, however, of the experiments reported in the third section of this paper it is probable that other toxic conditions exist in flax which may also be related to resistance.

III. Flax Extracts Toxic to Fungi

Although it had been demonstrated that under certain conditions flax extracts retarded the growth of *Fusarium lini* in culture (Reynolds, '24), it was desired to determine what concentration, if any, might prevent the growth of the fungus. Furthermore, as stated in the foregoing section, it was desired to study the effect of the cyanide content of flax upon the fungous growth. Hence water extracts of both fresh and dried flax were tested as regards toxicity to *Fusarium lini*. These extracts were made by grinding the flax and steeping it in water, usually over night. Various methods of filtering and the effects of these upon the toxic quality of the extracts were tested. None of these methods used prevented the characteristic toxic effects. To the filtered liquid were added salts and glucose in the same proportions as used in the standard check medium (A) throughout this study. The formula for this was water 1000 cc., magnesium sulphate 2 grams, later reduced to 1 gram, calcium acid phosphate 1 gram, potassium nitrate 10 grams, and dextrose 20 grams. A portion of each flax extract thus provided with standard quantities of nutrients was autoclaved at 15–20 pounds pressure for twenty minutes; and a corresponding portion filtered through bacteriological filters. At first the Berkefeld and Mandler filter cylinders were used, but the Seitz filter was later adopted for speed and convenience. Sterilized pipettes and culture chambers provided means of transferring the sterilized medium when necessary.

Through the kindness of Dr. E. C. Stakman seven of the strains of *Fusarium lini* with which Broadfoot ('26) carried on his experiments were made available. These were kept in culture and used during the course of the work. They were numbered from 2 to 8 approximately in their general decreasing order of pathogenicity, although it is clear that when several strains of flax are tested the flax strains and those of the fungus can not be arranged in a simple series in relation to one another. Those having the

higher numbers, especially 7 and 8, grew more abundantly in culture media than those having the lower numbers. Many hundreds of cultures were made and the dry weight of growth produced in fourteen days was determined. In nearly all cases identical triplicate cultures were run and averaged for the dry weights. Checks on the standard medium were carried with each set of new inoculations. Flax extracts were made from nearly all of the flax varieties and conditions of growth which were tested for hydrocyanic acid as reported in the preceding section. At the same time that each strain of flax was being tested for HCN content, culture series were run, using both fresh extracts and the extract which had been aerated and hence deprived of most of the HCN. The percentage dry weight of each sample of flax was determined before it was used in the HCN and culture studies. This was necessary since different ages and conditions of growth were being tested. In the course of the aeration process foaming sometimes took place, and at different times diphenyl ether, amyl alcohol, and caprylic alcohol were used to break the foam. Each of these was tested a number of times in different ways as to its effect on the quantity of growth of the fungus. Neither amyl alcohol nor diphenyl ether showed any repressive effect on the fungus and the very dilute quantities used did not stimulate growth. Caprylic alcohol proved to be extremely toxic so that one or two drops in a 50-cc. culture prevented growth completely.

In table v a summary of a considerable number of representative experiments is given. The standard concentration of flax extract was 9 parts water to 1 part dry weight of flax. From .2 to .3 of a gram was the usual dry weight of mycelium produced in the standard check medium A. It was soon evident that dilute flax extracts, that is, below one-half standard strength prepared as stated above, usually stimulated the growth of this *Fusarium*. From .3 to .5 of a gram of growth was usual and the higher the concentration of flax extract, up to certain limits, the greater was the mycelial growth. This favorable effect of the flax medium can probably be ascribed to the added nutritive materials from the flax. Fresh, green flax and dry flax powder

TABLE V

RESULTS OF CULTIVATION OF F. LINI IN FLAX EXTRACTS

Exp. No.	Flax No. and height in inches	Concentration	Filtered (F) Autoclaved (A)	Previous treatment	Result	Remarks
B # 1	# 6—3½	1	A	Powdered	O	Shoot*
B # 1	# 6—3½	1/4–1/2	A	Powdered	<	Shoot
B # 1	# 6—3½	1/2–1	F	Powdered	O	Shoot
B # 1	# 6—3½	1/4–1/2	F	Powdered	< S	Shoot
B # 2	# 2a—2½	1/4–1	A	Powdered	<	Entire
B # 2	# 2a—2½	1/4–1	F	Powdered	<	Entire
B # 3	# 7—7	1/4–1	A	Powdered	<	Entire
B # 3	# 7—7	5/12–1	F	Powdered	O	Entire
B # 3	# 7—7	1/4–1/3	F	Powdered	G	Entire
B # 4	# 5—12	1/4–1	A	Powdered	G	Leaves
B # 5	# 5—12	1/4–1	A	Powdered	G	Stems
B # 6	# 11—6	1	A	Powdered	O	Leaves
B # 6	# 11—6	1/4–1/2	A	Powdered	G	Leaves
B # 6	# 11—6	1/4–1	F. A.	Powdered	G	Leaves
B # 7	# 11—6	1/4–1	F. A.	Powdered	G	Stems
17	# 2—2	00	F	Powdered	G >	Roots
18	# 3—6	1/3	F	Powdered	G	Roots
22	# 5—8	1/4	F. A.	Aerated	G	Shoot
22	# 5—8	1/20	F. A.	Aerated	G >	Shoot
22	# 6—7	1/3	F		O	Shoot
22	# 6—7	1/3	A		G	Shoot
22	# 6—7	1/12	F		G	Shoot
22	# 6—7	1/12	A		G >	Shoot
30	# 1	1/8	F	Aerated	G	Shoot
35	# 7	1/10	F	Aerated	G	Shoot
36	#4—6	1/8	F. A.	Aerated	G	Whole
38	# 6—6	1/20	F	Aerated	G	Less than in Exp. 36
39	# 2—8	1/16	F. A.	Aerated	G	
41	# 1—2½	1/14	F. A.	Aerated	G	
53	# 6	1/7 & 1/28	F		G	Fresh, green
54	# 2	1/7 & 1/28	F. A.	Aerated	G	Fresh, green
54	# 6	1/6 & 1/24	F. A.	Aerated	G	Fresh, green
58	# 3—7½	1/10–1/40	F		G-M	Fresh, green
58	# 3—10	1/15–1/60	F		G-M	Fresh, green
63	# 6—10	1/10–1/20	F. A.		G >	Fresh, green
65	# 2—10	1/8–1/16	F. A.		G >	Fresh, green
67	# 1 & 2—8	1/17	F. A.		G	Fresh, green
71	# 7—4	1/14	F. A.		G-M-S	Fresh, green
72	# 3—4	1/10–1/20	F. A.		G	Fresh, green
78	# 4 & 6—4	1/16–1/32	F. A.		G	Fresh, green
80	# 2—4	1/10–1/20	F. A.		G	Fresh, green
81	#2 & 7	2, 1, 1/2	F. A.		O-G	Fresh, green
84	#1 & 4–1½	1/5	F. A.		G	Fresh, green
86	#2 & 5—5	1/4	F. A.		G	Fresh, green
88	#1—3 & 20		F. A.		G	Fresh, green
92	# 1 & 6—3	1/4 & 1/7	F		G	Fresh, green
95	# 2 & 7	2, 1, 1/2	F. A.		O	
98	# 1 & 6—4	1/3–1/6	F. A.		G	Fresh, green
101	# 7—10	2/3–1/3	F. A.		G >	Roots & shoots separate

TABLE V (*continued*)

Exp. No.	Flax No. and height in inches	Concentration	Filtered (F) Autoclaved (A)	Previous treatment	Result	Remarks
117	# 11—10	1	F		G	Dry roots only
121	# 4—	1	F		O	Dried flax powder
123	# 1a—3	1	F		O	Fresh, green flax
127	# 4—	1	F		O	Dry, powdered

*—Unless definitely stated the extract was made from powdered flax.
M—About equal to growth on Med. A.
O—No growth of fungus developed.
S—Small growth, less than on checks in Med. A.
G—Good growth, distinctly better than checks on Med. A.
>—Weight of fungus decreasing with dilution of extract.
OO—Diluted 50–100 times.
<—Weight of fungus increasing with dilution of extract.

have both been used in preparing these extracts, as illustrated in the following tabulations of results from a few experiments.

Experiment 71—73.2 gms. # 7 fresh green flax to 1000 cc. water

	Filtered		Autoclaved	
	# 2 *F. lini*	# 7 *F. lini*	# 2 *F. lini*	# 7 *F. lini*
Full str. extr.	.2975	.3707	.2160	.3619
Half str. extr.	.2286	.2354	.2716	.3482
Checks # 71 & # 72	.2442	.2885		

Experiment 72—106.5 gms. # 3 fresh green flax to 1000 cc. water

Full str. extr.	.3147 (.0705)*	.3931 (.1046)	.2977 (.0535)	.3697 (.0812)
Half str. extr.	.2782 (.0340)	.3323 (.0438)	.2814 (.0372)	.3302 (.0417)

*The numbers in parentheses show increase in growth in flax extract over the growth in the check medium.

Experiment 78—61.45 gms. # 4 fresh green flax to 1000 cc. water

Full str. extr.	.2832	.3160	.2569	.3079
Half str. extr.	.2687	.3043	.2579	.2905

77.14 gms. # 6 fresh green flax to 1000 cc. water

Full str. extr.	.3045	.3323	.2973	.3318
Half str. extr.	.2704	.2871*	.2665	.3017
Checks	.2179	.2218		

* Had been contaminated and refiltered before inoculation.

Experiment 80—94 gms. # 2 fresh green flax to 1000 cc. water

Full str. extr.	.2771	.3033	.2850	.3048
Half str. extr.	.2973	.2623	.2799	.2817
Checks	.2179	.2218		

Experiment 81—102.0479 gms. air-dry # 2 flax powder to 500 cc. water. 102.2809 gms. air-dry # 7 flax powder to 500 cc. water. # 7 *F. lini* used

	# 2 Flax	# 7 Flax	# 2 Flax	# 7 Flax
Full str.	.0000	.0000	.0000	.0000
Half str.	.0000	.0000	.0000	.0000
Quarter str.	.5013	.5149	.5041	.5156
Checks	.2409			

The first four experiments tabulated are typical of many which were carried concurrently with those in which the determinations of cyanide content were made. It will be noted that # 2 *F. lini* consistently produced less growth than # 7 on all flax extracts and the check medium. In Experiment 72 a difference in growth between the check cultures and the flax extract cultures shows that the # 2 *F. lini* made less increase of growth than did # 7 *F. lini*. This would indicate that the former strain can make less use of the added nutritives from the flax or else is retarded more by the flax extract than the latter. It seems that the first alternative is more probable since the # 2 *F. lini* does not make as good use of the nutritives in the check medium as does # 7, and at these concentrations of flax extract a retarding action is not evident. Autoclaving seems to have little effect in changing the nutritive qualities of the flax media at these concentrations, for the differences between the growth in the autoclaved and the filtered media are neither great nor regular. In Experiment 80, since there is more flax per liter than in Experiments 71 and 78,

we should expect a larger fungous growth. However, in the main this is not true. It is possible that a slight toxic effect is exhibited here, but certainly the figures are not clearly significant. Experiment 81, however, exhibits a clear case of toxicity for the half- and full-strength flax cultures and as clearly indicates that the quarter strength is more than twice as effective as the check medium in producing growth. This latter concentration corresponds with the half-standard strength. The juice expressed from fresh flax, when used as above without dilution, also prevented growth of the fungus. In different varieties of flax it was found that various concentrations prevented the growth of *F. lini*. It was necessary therefore to attempt to determine the minimum concentration of flax extracts of different varieties which would just prevent growth of the fungus.

Experiment 147 will illustrate the procedure. Dry flax powder (# 3 flax), weighing 25.34 gms., was steeped with 100 cc. of distilled water for two days. The liquid was pressed out and more water added with successive pressings until 250 cc. of flax extract had been obtained. Since a small meat press was used some water was left in the flax material and the total water added was somewhat more than the 90 per cent, which has been used as the arbitrary standard. The salts and glucose were dissolved in the standard proportions and the medium was then filtered through the Seitz bacteriological filter. With sterile, graduated pipettes a series of cultures was made as follows, and designated "Series A":—Three tubes of Check Medium A, marked O; three tubes of full-strength extract, marked 1; three tubes with 9 cc. of extract, and 3 cc. of Medium A, marked ¾; three tubes with 6 cc. of extract and 6 cc. of Medium A, marked ½; and three tubes with 3 cc. of extract and 9 cc. of Medium A, marked ¼. These were left several days in the incubator to test for freedom from contamination and then inoculated with # 7 *Fusarium lini*. Six days after inoculation there was no growth except in the checks (0). At this time one set, from 0–¼, was reinoculated and designated as X. A second set was filtered into fresh tubes, autoclaved, inoculated, and designated Y. A set Z, made up as follows from the third tube of full-strength flax extract of the original set A, was autoclaved and inoculated:—

(1) 2.0 cc. of full-str. extract and 8.0 cc. of Medium A
(2) 1.5 cc. of full-str. extract and 8.5 cc. of Medium A
(3) 1.0 cc. of full-str. extract and 9.0 cc. of Medium A
(4) 0.8 cc. of full-str. extract and 9.2 cc. of Medium A
(5) 0.6 cc. of full-str. extract and 9.4 cc. of Medium A
(6) 0.4 cc. of full-str. extract and 9.6 cc. of Medium A
(7) 0.3 cc. of full-str. extract and 9.7 cc. of Medium A
(8) 0.2 cc. of full-str. extract and 9.8 cc. of Medium A
(9) 0.1 cc. of full-str. extract and 9.9 cc. of Medium A

Nine days after inoculation no fungous growth was present in sets X and Y except in the ¼-strength culture of Y, where the inoculum had become lodged at the surface of the liquid and a slight growth had developed. In set Z some growth had taken place in all the tubes with evidently much less in (1) and (2). Thus the limiting toxicity for complete inhibition was at ¼ strength for the autoclaved material, although a retarding action was manifested in the second tube of set Z. In the filtered extract the toxic limit for complete inhibition was below the ¼ strength as seen in set X.

In Experiment 102 # 3 flax powder made up one-third standard strength prevented the growth of # 7 *F. lini*. In Experiment 121 one-half strength flax extract from # 4 flax also prevented the growth of the same strain of fungus. Full-strength and one flask of half-strength extract from # 1 flax prohibited growth of # 7 *F. lini*. In this experiment (# 123) fresh, green flax, 3–3½ inches tall, from the outdoor garden was used. Both the inhibiting strengths had a pH of 3.53. A second flask of the half-strength medium produced a growth of .5087 gms., and an average of three cultures in the quarter strength was .3816 gms. The extra-large growth in the one tube of half-strength medium suggests a toxic stimulatory action. Other occasional results of this irregular nature have been noted, especially at or near the point of complete inhibition. Thirty grams of # 4 flax powder in 300 cc. of water were used in Experiment 138, from which a series of dilutions was made as follows: 1/5, 2/5, 3/5, 4/5, 9/10, and 5/5 strength. A regular increasing gradation of growth from the 3/5 concentration downwards, a slight growth in the 4/5, and none above indicates the approximate inhibiting concentration of this material. A considerable number of the early experiments performed in eastern New York indicated a completely inhibiting toxicity of the flax extracts at or below the standard strength, as

illustrated in the summary given above. These included both extracts from dry flax powder and extracts from fresh, green flax plants. When this work was continued at the Missouri Botanical Garden it was found that some flax powders gave this same inhibiting result, while others allowed abundant growth of *Fusarium lini* at this concentration. This was true even when inoculations were made from the same fungous culture at the same time for different flax extracts. After several series of cultures had given these conflicting results a careful check was made of the sources of the various flax powders used. It was found that uniformly those powders derived from plants grown in New York gave the inhibiting action, while those from plants grown at St. Louis failed to show this degree of toxicity. This interesting phase of the problem will be studied further, when a new supply of flax powders from several climatically different regions is available.

It is evident from these experiments that different strains of flax possess the toxic quality in different proportions and that different environmental conditions surrounding the flax determine the concentration in the same strain at different times.

The effect of heat on the toxic material of flax was tested in two ways. Flax powder was autoclaved for one hour at 18 pounds pressure and used for extracts. No growth of *F. lini* occurred in this extract at standard concentration. Inhibiting flax extracts of standard concentration have been autoclaved and tested for growth of the fungus. In most experiments the fungus was still prevented from growing, but occasionally growth developed. The latter cases have been interpreted as indicating a degree of toxicity close to the margin of inhibition and a partial injury of the toxic material by autoclaving in liquid medium.

Efforts were made to eliminate the toxicity by aeration and to transfer such toxicity to Medium A by aerating flax extracts into this medium. The latter experiment was entirely negative in results and the former essentially so. Fresh, green flax of the # 11 strain was ground and aerated twice for a total of about thirty-six hours. The HCN content was .243 per cent. The residual liquid, 240 cc., was provided with the usual salts and glucose in proper proportion and used as a culture medium for # 7 *F. lini*. A retarded development took place in two filtered

cultures and no growth in the third, while the three autoclaved flasks developed a good growth. Since this medium was somewhat less than one-half standard strength it seems improbable that the aeration had any appreciable effect on its toxicity. In Experiment 102, 39 gms. of # 3 flax powder, after a preliminary steeping in water, was aerated into 160 cc. of water, to which the salts and glucose were added to convert it into a standard Medium A plus any volatile products from the flax. This was made into three 50-cc. cultures and called A. The flax filtrate of 1000 cc., obtained after the aeration, was divided into several portions. From 500 cc. of it 280 cc. were distilled into a 5 per cent KOH solution for a cyanide determination. This portion (B) was then made up to its original volume; a portion (C) was retained unchanged; and a portion (D) was diluted to half strength. All these were prepared as usual as cultures and one half of the B, C, and D flasks were autoclaved, while the other half were used as filtered material. The following were the growth results in grams from inoculation with # 7 *F. lini*:—A, .3066; B-filtered, .5220; B-autoclaved, .4339; C-filtered, no growth; C-autoclaved, .5681; D-filtered, .4515; D-autoclaved, .4588. Autoclaving again seemed to remove the toxic effect. No toxic effect was noted in the Medium A, thus indicating that no volatile toxin was present in appreciable quantity. The process of distillation, however, destroyed the toxic condition as seen by comparing B and C. The original concentration of flax extract was about one-third standard and when diluted to half strength fell well below the inhibiting concentration as seen in the D cultures. Autoclaving makes little difference in this case.

It was thought that perhaps the toxic material was a dialyzable compound. The following experiments were therefore made to test this hypothesis. For the first experiment (# 121) 47 gms. of # 4 flax powder were left in 450 cc. of water for two days and then, after filtering, washed with several portions of water and pressings in a Buechner funnel with suction until a total of 1000 cc. of filtrate were obtained. Four hundred cc. were then dialyzed through a collodion bag, made as usual in a Kjeldahl flask. An electric stirrer was fitted into the neck of the bag, by a rubber stopper, and dialysis for two days in running tap-water was

followed by one more day in running distilled water. There were 500 cc. of liquid present at the end, to which salts and glucose were added. Fifty-cc. flask cultures were made from this and also from the remainder of the flax filtrate which had not been dialyzed, using as always salts and glucose to equal their concentration in Medium A. Half of the flask cultures were autoclaved and half filtered. None of the cultures from the filtered, original filtrate produced growth; the autoclaved, filtrate cultures produced an average growth of .5923 gms.; the dialyzed, autoclaved cultures produced .2177 gms. of fungous growth; and the filtered, dialyzed cultures had an average of .2634 gms. of fungus. As this flax extract was less than half-standard strength and during the process of dialysis considerable precipitation had taken place in the bag it was thought best to check the results carefully. In Experiment 132, 26 gms. of # 6 flax powder were used, since the # 4 flax was exhausted. The dialyzing was continued with stirring for three days with a total of 260 cc. liquid left at the end. This was filtered with suction and used without dilution as a culture medium. No growth took place in the filtered series of flasks, but a belated growth of fungus did develop in two out of the three of the autoclaved series in a twenty-day period. No numerical results were taken. If dialysis removes any of the toxicity, as seemed to be the case in the first experiment, it is not rapid nor very effective as seen from the results of the second experiment. In the latter test the filtrate was of standard strength and even in the autoclaved series still showed some toxicity both by the belated development and by the development of growth in only two out of the three flasks. Further dialysis experiments are planned covering several strains and ages of flax.

The relative effects of root and shoot extracts were tested out by an experiment (# 101) as indicated in table VI.

No toxic effects are evident at the concentration used and the root extracts provided less nutriment than those from the shoot. The greater growth of # 2 *F. lini*, as compared with # 7 *F. lini*, in the shoot cultures may indicate an ability to use flax extractives to better advantage, or possibly an ability to withstand a certain toxic action which, although not sufficient to reduce the growth of # 7 *F. lini* below the check culture, nevertheless holds back this

TABLE VI

	# 7 *F. lini*		# 2 *F. lini*	
	Filtered	Autoclaved	Filtered	Autoclaved
Root				
Full str.	.5611		.5472	.6714†
Half str.	.4057	.4580*	.3957	.4773*
Shoot				
Full str.	.7793		.8591	.8675*
Half str.	.4536	.4887*		.5775*
Checks	.2427		.2357	

* Average of 2 cultures. † One culture only.

latter fungus from making as full a growth as it should. The latter conclusion would be in accordance with the conclusion reached in Experiment 81. The greater growth of # 7 *F. lini* in the check medium, although numerically not clearly significant, is in line with all of the other cultural work with this strain as compared with # 2 *F. lini*.

It is interesting to note from these various experiments that dilutions of flax extract, which are only half as concentrated as the critical concentration for complete inhibition, often not only allow development of the fungus, but actually produce a much more abundant growth than the standard, check medium. Hence it appears that it is necessary for this toxic material to be in a rather concentrated form before it can overcome the nutritive stimulation of a culture medium favorable to the fungus. The range of concentration from that which gives greatest growth to that which is completely inhibitive is often quite narrow.

That the toxic effect is not due to acidity or alkalinity of the medium is evident from two considerations. First, the flax wilt fungus is able to grow well throughout a wide pH range. Second, the flax media which prevented growth ranged mostly from a pH of 3.56 to 3.60, with the extreme at 4.06, while the standard check medium was 3.52 and the flax medium which allowed prompt and abundant growth ranged as high as 5.00 to 6.35 when ready for inoculation. Autoclaving the flax medium sometimes raised the pH as much as a point, although at other times only a few tenths. At two different times in the course of this work abundant growths

of foreign fungi were found in flax media which completely prevented the growth of *F. lini*. In each case it was one out of three identical flasks which had become infected. One of these was *Monilia sitophila* and the other an *Aspergillus*. This indicates that the toxic material in flax is at least somewhat specific for the *Fusarium*, but many further tests must be made to determine the range of toxic effect upon fungi in general and plant pathogenes in particular. A test was made to determine the effect of the toxic substance upon the fungus by transferring a mass of mycelium from a flax extract in which it had failed to grow into a flask of Medium A. There was no growth from this inoculum, showing that the flax medium had killed the fungus, both mycelium and spores, and not simply prevented further growth.

ETHEREAL AND ALCOHOLIC FLAX EXTRACTS

Flax powders have been extracted with ether and with alcohol by the use of the Soxhlet apparatus and also by suction of the cold reagent through the plant material in a Buechner funnel. The following summary of such experiments will indicate the methods and the results. In Experiment 126 # 4 flax powder was extracted with ether in a Soxhlet apparatus until practically no green color remained to be extracted. The powder was dried and made into a standard flax extract. Flask cultures, made as usual, were inoculated with # 7 *F. lini*. Abundant growth resulted. Some of the same powder was in a similar manner extracted with alcohol and the extracted flax made into flask cultures. The fungus grew well. As a check some of the same powder was made into a culture medium without having been extracted with ether or alcohol. No fungous growth took place. Evidently the ether and alcohol treatments had removed the toxic principle, at least below the inhibitive concentration. The ether and alcohol extracts were evaporated to dryness, taken up in a small amount of hot water, filtered, and made into culture media. When inoculated with the fungus a very slow development took place in the culture from the ether extractive, showing, however, only after 12 days of incubation. No growth of *Fusarium* occurred on the culture from the alcoholic extractives, although a contamination of *Monilia sitophila* took place and grew vigorously. Evidently

at least a portion of the toxic quality was transferred from the flax by means of the ether and the alcohol and hence is soluble in these reagents. In another experiment an alcoholic extract was made by treating the powder in a Buechner funnel with successive portions of alcohol, and applying suction. The extract was then evaporated to dryness, taken up in hot water, filtered and made into flask cultures. After inoculation with the fungus and incubation for eleven days with no growth resulting, a re-inoculation was made and no growth was developed. Another ether extraction experiment was tried in which the resulting extract was concentrated and then added to a series of tubes of Medium A. The first tube has 1/10 cc., the second 2/10 cc., etc., up to 5/10 cc. Apparently no growth took place in the more concentrated tube, a slight growth in the next lower, and so on down to the least concentrated tube in which a good growth took place, although not as rapid or abundant as in the check Medium A. Exact studies of the concentration limits for these extracts have not been made. In some few cases ether extractions have failed to produce toxic cultures, but lack of proper material has made it impossible to determine the cause of these failures, although it is suspected that it is associated with the question of the source of the flax powder. Attempts to obtain a crystallized product from these ether and alcohol extracts from rather small quantities of flax have so far failed, but will be renewed when there is a sufficient supply of dry flax of known toxicity available.

General Discussion and Conclusions

In experiments of the kind reported here there are two factors acting counter to one another. In the plant extract there are definite food values which are added to those included in the nutrients of the check medium. These would tend to increase the total growth of the fungus over the amount in the check cultures. The toxic quality must be sufficiently strong to overcome the nutritive value of the entire culture medium. In Fermi's solution used in the first studies (Reynolds, '26) the glycerine is a poor source of carbon for this fungus. Glucose, used in the check medium in the studies reported here, is a very satisfactory source of carbon. Thus while the dry weight of fungus in Fermi's

solution was usually less than 100 mgs., that in Medium A was usually from 200 to 300 mgs. The more dilute flax extracts used in the first study definitely retarded the growth of *Fusarium* in Fermi's medium, although no complete inhibition was found. In the more favorable Medium A the dilute flax extract served mainly to add nutrients and the toxic quality was thus masked. In the more concentrated flax media, from one-half to full strength, the toxic quality was evidently strong enough under some circumstances to over-balance completely the nutritive values and even kill the fungus. Since in many experiments the flax material was left long enough for the linase to hydrolyze at least a large part of the glucoside, linamarine, into HCN, acetone, and glucose, and the autoclaving produced sufficient heat to drive off the volatile toxic materials, it is clear that there must be in the flax a second toxic substance. In most of the experiments autoclaving of the flax extract did not so reduce its toxicity that the fungus could grow at the standard strength, yet occasionally at somewhat less than such a concentration the autoclaved medium did allow some growth. At about half-standard concentration autoclaving so reduced the toxicity that the added nutritive materials of the flax extract caused a distinctly larger fungous growth than in Medium A. At still lower concentrations autoclaving had little effect on the quantity of growth. It seems probable then that this second toxic material is somewhat thermostable although not completely so. Its other characteristics, as brought out in the experiments reported, are solubility in water, ether, and alcohol, and its essential non-volatility. It is somewhat specific for certain fungi and seems not to greatly influence others. While it does not dialyze readily it is not a coagulable protein. Its toxicity is rather low but when in sufficient amount it is absolutely deadly to this *Fusarium*.

Very little information appears in botanical writings concerning any such toxicity as reported here. Osterhout ('25) has reported that cells of *Valonia macrophysa* placed in sap extracted from similar cells quickly die. This result he attributed to the contrasting salt concentrations, but he did not eliminate the possibility that early death was due to other toxic factors. Prát ('27) heated extracts of several plant tissues and showed that living

cells from the same tissues die more quickly in these extracts than in isotonic sea-water of similar pH or in tap water. This seemed to indicate a special toxic action. O'Connor ('27) reported that specific, inhibiting, diffusible substances from plant and animal tissues, named by him "speciamines," inhibited growth of pollen tubes of "foreign pollens." Newton and his associates ('29) have noted an inhibiting action of wheat leaf filtrate on the germination of urediniospores and have suggested that phenolic substances are responsible for this action. It is probable that some of these observations, especially the last two mentioned, are in the same category as those reported for flax extracts.

Several materials known or supposed to exist in plants or in plant extracts should be considered in an attempt to determine the chemistry of the toxic material. Since formaldehyde may be formed from chlorophyll under certain circumstances (Warner, '14) and has been reported in the sap of green plants (Angelico and Catalano, '13), and since the flax extracts contain much leaf pigment it was thought that possibly toxicity might be ascribed to this compound. Tests were made for formaldehyde in these extracts but with entirely negative results. Mazzetti ('28) has shown that although boiled linseed oil develops bactericidal properties, these do not appear in the raw product from flax, almond, soybean, and castor bean. Since toxicity is found in unheated flax extracts, oil of the character of those named cannot be responsible for the inhibiting action. High relative acidity, coagulable proteins, and dialyzable substances have apparently been eliminated by experimental results. Toxic phenolic substances, such as suggested by Newton and his associates ('29), have not been specifically studied.

The different degrees of parasitism as shown by Broadfoot ('26) to exist in the different strains of *F. lini* should be considered in relation to resistance. In one experiment (# 101) # 2 *F. lini*, which in Medium A and dilute flax cultures produced less growth than # 7 *F. lini*, gave a much greater growth, especially in shoot extract which has been shown to be strongly toxic. It appears that the greater virulence of # 2 *F. lini* is associated with its ability to resist the toxic effects of flax, rather than with any special adjustment to nutritive qualities of its host.

That resistance to flax wilt and the toxicity of flax extracts may be associated phenomena is suggested not only by the more or less specificity of these extracts for *Fusarium lini*, but also by the relation of both phenomena toward changes of environment. It has been a rather common belief among those who have worked considerably with flax that it is easily influenced by the environment. Several specific evidences are discussed by Armstrong and Eyre ('12), and in the study of varietal distribution of HCN this apparent environmental effect was noted. The degree of toxicity of flax extracts seems also to vary with the environment surrounding the growing flax as evidenced by the markedly lower toxicity of the extracts from flax grown at St. Louis as contrasted with those from flax grown in New York. Resistance to flax wilt is known to vary with temperature as noted by Tisdale ('11) and studied by Jones and others ('26) more in detail, and it is possible that other environmental factors also are important.

It is realized by the writer that a number of important suggestions made here must be much more carefully studied, but the general trend of this investigation seems to be well established and details will be studied further as time will allow.

SUMMARY

A general discussion of some of the essential problems in the physiology of plant disease is given, with emphasis upon the need of attacking them as a special project, with the active coöperation of variously trained specialists, rather than merely as an incident in routine pathologic investigations.

By cultural studies two kinds of toxic substances are recognized in flax extracts.

The glucoside, linamarine, producing HCN upon hydrolysis, has been discussed previously. Numerous analyses for HCN in flax extracts show a great variability of amount of this glucoside: its probable presence in larger amounts in the more resistant strains of flax; and its apparent close association with the young, actively functioning cells.

A new, somewhat thermostable, toxic material appears in the flax extracts of higher concentration. This material is apparently non-dialyzable, soluble in water, ether, and in alcohol; and varies

in quantity both in relation to environmental factors and in relation to variety of flax. In many extracts it is completely inhibitive to *Fusarium lini* at the normal concentration of the flax juice.

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THE GENETIC ANALYSIS OF AN UNUSUAL RELATIONSHIP BETWEEN SELF-STERILITY AND SELF-FERTILITY IN NICOTIANA[1]

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A number of crosses between *Nicotiana alata*, *N. Sanderae*, and *N. Langsdorffii* were grown at the John Innes Horticultural Institution from 1923 to 1926 by the junior author. In most of these crosses the inheritance of self-sterility was quite straightforward, and the results obtained were substantially the same as those reported by East ('19), and East and Yarnell ('29). That is, the first generations consisted entirely of self-fertile plants, but on crossing the F_2 plants *inter se*, self-sterility reappeared in certain of the second-generation families. Among the plants of *N. alata* there was, however, a single plant (No. 15-2) whose cross-sterility relationships were exceptional and which gave rise to exceptional families when crossed with *N. Langsdorffii*. It is with the behaviour of No. 15-2 and its progeny that this paper is concerned. The results obtained from 1923 to 1926 with these plants are presented graphically in fig. 1, where they are contrasted with the results normally obtained upon crossing *N. alata* and *N. Langsdorffii*. Attention is called to the following peculiarities of the cross with No. 15-2: (1) The original exceptional plant was incompatible as a female with *N. Langsdorffii* though compatible as a male; (2) The F_1 was composed of self-sterile and self-fertile plants in approximately equal numbers—it will be remembered that normally in crosses between *N. alata* and *N. Langsdorffii* self-sterility would not appear until the second generation; (3) The self-sterile F_1's were compatible both as males and females with their self-sterile parent species *N. alata*, while with *N. Langsdorffii* they were compatible as males but incompatible as females.

[1] Much of the work reported in this paper was carried on under a National Research Fellowship in the Biological Sciences.

That is, they were *cross-sterile with their self-fertile parent and cross-fertile with their self-sterile parent.* No. 15-2 gave similar exceptional results when crossed with another self-fertile strain of tobacco, an ornamental garden variety of unknown ancestry obtained from Mr. E. A. Bowles. It bore small dark red flowers and was probably a self-fertile segregate from the cross *N. Langsdorffii* × *N. Forgetiana.*

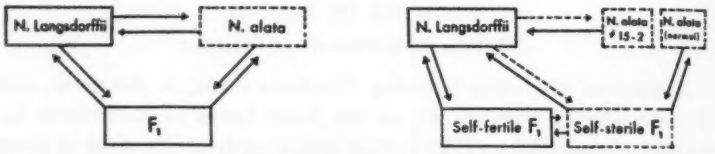

Fig. 1. Sterility and fertility relationships in normal crosses (left) and in hybrids with *N. alata* No. 15-2 (right). Solid lines indicate self- or cross-fertility; dotted lines, cross- or self-sterility.

All of these complications can be explained if we assume that No. 15-2 carried one self-sterility allelomorph of a slightly different nature from those normally present in *N. alata. Their* behavior is outlined by East and Yarnell ('29) as follows: "The action of these allelomorphs was such that a plant of constitution S_1S_2, when pollinated by pollen from a plant of constitution S_2S_3, produced two types of progeny, S_1S_3 and S_2S_3, due to the slow growth of the pollen tubes bearing the factor S_2." Continuing the notation developed by East and Mangelsdorf ('25) and East and Yarnell ('29), we may designate the exceptional allelomorph in No. 15-2 as S_F. This allelomorph operates like those designated by East and his students, but has the additional property of inhibiting the growth of pollen carrying the full fertility allelomorph S_f as well as that carrying S_F. Plant No. 15-2 was heterozygous for S_F and would set no seed with any pure self-fertile plant, since such pollen (all carrying S_f) would not grow fast enough to cause fertilization.

The other allelomorph of 15-2 was a normal self-sterility allelomorph. In the absence of precise tests with East's material we cannot tell just which one it was but may designate it S_n to indicate any one of the self-sterility allelomorphs. No. 15-2 is therefore represented in fig. 2 as of the genetic constitution S_FS_n.

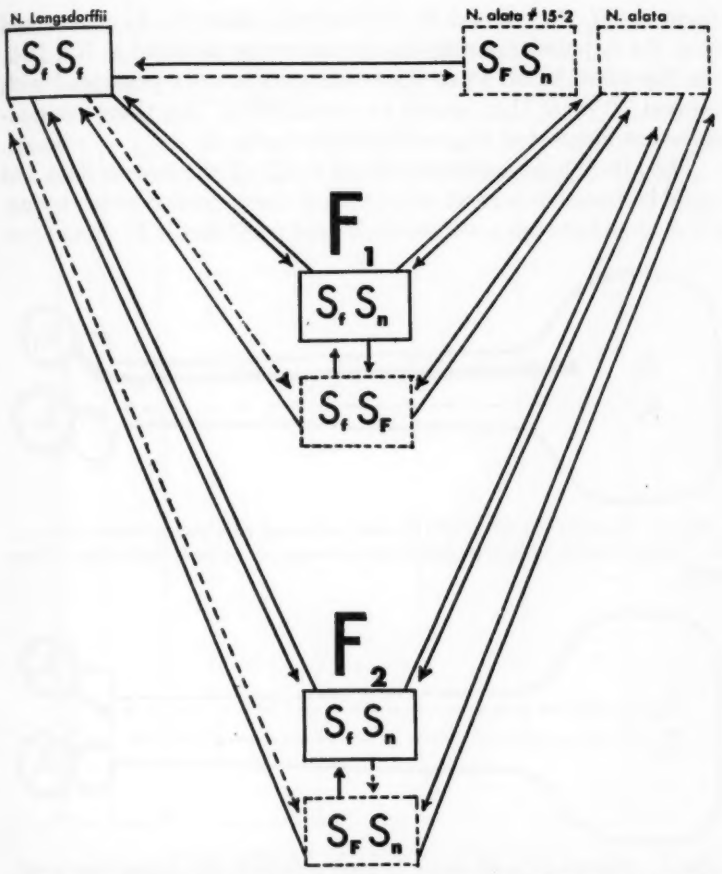

Fig. 2. Diagram showing the factorial analysis of the cross with *N. alata* No. 15-2. Solid lines indicate self- or cross-fertility; dotted lines, self- or cross-sterility.

When the pollen of 15-2 was applied to the stigmas of *N. Langsdorffii* (S_fS_f) both kinds of *alata* pollen (S_F and S_n) would affect fertilization, giving an F_1, half of which would be of the constitution S_fS_n, and half S_fS_F. The first would be self-fertile though carrying a self-sterility allelomorph (S_n). The second class would be self-sterile though carrying a self-fertility allelomorph (S_f). Furthermore, when pollinated with their self-fertile parent, *N.*

Langsdorffii, they would be cross-sterile, since the S_F gene would stop the S_f pollen precisely in the same way as it did in No. 15-2. On the other hand, when these self-steriles were pollinated with normal *N. alata* they would be cross-fertile. All these relationships are illustrated diagrammatically in fig. 2.

The above explanation therefore fitted all the known data and could be tested in several ways; four of these seemed worth trying. (1) A cross between a self-sterile F_1 and a self-fertile F_1 should give

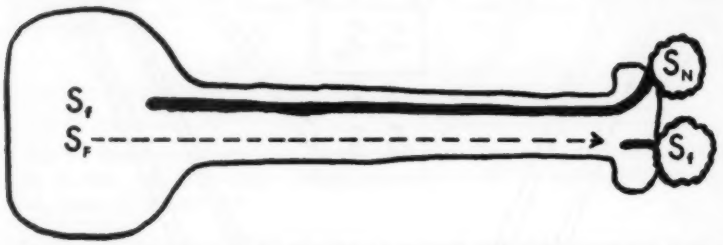

Fig. 3. Diagram of a self-sterile F_1 pistil pollinated with pollen from a self-fertile F_1. Dotted line shows antagonism between factors of the style and pollen. Cross fertile.

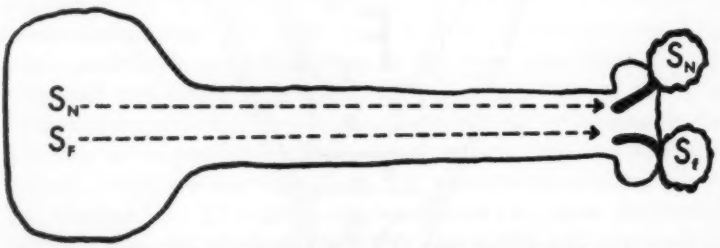

Fig. 4. Diagram of a self-sterile F_2 pistil pollinated with pollen from a self-fertile F_2. Dotted line shows antagonism between factors of the style and pollen. Cross sterile.

self-fertiles and self steriles in the F_2. (2) Though the self-sterile F_1's had been cross-fertile when pollinated by their self-fertile siblings the F_2 self-steriles should be cross-sterile when pollinated by their self-fertile siblings. These relations are diagrammed in figs. 3 and 4. Family 5N-30 (the result of pollinating self-sterile F_1, No. 29-12, by its self-fertile sibling No. 29-17) was grown to test these hypotheses. They were completely verified as shown in

TABLE I

POLLINATIONS ON SELF-FERTILE PLANTS

Plant No.	Fertile Matings			Sterile Matings		
	Self-pollinated	Pollinated by self-fertiles	Pollinated by self-steriles	Self-pollinated	Pollinated by self-fertiles	Pollinated by self-steriles
1	4					
2	4					
3	4					
4	4					
5	2					
7	4					
8	4					
9	3					
11	4					
12	3			1		
13	4					
18	4	4	2			
19	2			1		
20	2					
21	4					
22	2					
24	4					
25	3					
26	4					
27	4					
29	4					
30	4					
31	3					
32	4					
33	4					
35	4					
37	1					
38	4					1
40	2			2		
41	2					
42	4					
43	3					
44	3					

POLLINATIONS ON SELF-STERILE PLANTS

6			1	4		
10				3		
14			1	5		2
15				7	16	13
16		2	1	8	5	2
17		2	3	4	5	11
23				3		
28		5	7	7		11
34				4		
36				3		
39	1			3		1
45				4		
46				4		
49				4		

table I. There were thirty-four self-fertiles and fifteen self-steriles. The self-steriles were furthermore not only cross-sterile when pollinated *inter se*, but were also cross-sterile when pollinated by their self-fertile siblings as is set out in the table.[1] (3) The self-sterile plants of the second generation, since they were carrying the factor S_F, should be sterile when pollinated by *N. Langsdorffii*. This prediction was also realized. Two unrelated strains of *Langsdorffii* were grown for the purpose. One, Family 1N-30, was from the same strain which had previously been used in the experiments. The other, Family K-30, consisted of six plants obtained from Kew Gardens. Both strains gave identical results. Both were fertile when pollinated with the F_2 self-steriles and both were cross-sterile when their pollen was used *on* the self-steriles. The data are summarized in table II. (4) The self-sterile F_2

TABLE II

RESULTS OF POLLINATING SELF-FERTILE AND SELF-STERILE F_2'S WITH N. LANGSDORFFII

	Fertile matings	Sterile matings
Self-fertile		
No. 2	4	0
No. 38	4	0
Self-sterile		
No. 14	0	1
No. 15	0	3
No. 16	0	4
No. 17	0	4
No. 28	0	6
No. 36	0	1

plants on the above hypothesis should (like the original *alata* grandparent No. 15-2) be heterozygous for S_F. Like it, therefore, when their pollen was used upon *N. Langsdorffii* they should yield progenies composed of self-sterile and self-fertile plants in approximately equal numbers ($S_fS_f \times S_FS_n \rightarrow S_FS_f + S_fS_n$). The appropriate pollinations were made at the John Innes Horticultural Institution, and several families were grown at the Missouri

[1] It must be remembered, of course, that Family 5N-30 was an F_2 from a very "wide" cross, and that, due to the recombination of modifying factors, cross- and self-incompatibility relationships could not be as clear cut as they would be in F_1, or in back-cross families. This whole matter is discussed below under the heading of "Modifying Factors."

Botanical Garden and at Washington University during the winter of 1930–1931. The results conformed completely with expectations and are briefly summarized in table III. With these results

TABLE III

RESULTS OF CROSSING N. LANGSDORFFII AND F_1 SELF-STERILES

Number of self-fertile plants	18
Number of self-sterile plants	8

the interpretation given on page 98 is thought to be well-established, and no further work is planned with this material. One additional test which *might* have been tried may be pointed out, since it indicates the complexities introduced by the factor S_F. A cross between a self-fertile F_2 and a self-sterile F_2 should give all self-steriles, in two intra-sterile, inter-fertile classes ($S_fS_n \times S_FS_n = S_fS_F$ and S_nS_F). A cross between these classes should produce both self-fertiles and self-steriles. In other words, on inbreeding, S_F produces a bewildering maze of interweaving classes in which self-sterility seems to be dominant to self-fertility, and self-fertility dominant to self-sterility, as the following examples show:

$$\text{S. fert. } S_fS_f \times \text{S. ster. } S_FS_n \longrightarrow \text{S. fert. } S_fS_n \;\&\; \text{S. ster. } S_fS_F$$

$$\text{S. fert. } S_fS_n \times \text{S. ster. } S_FS_n \longrightarrow \text{S. ster. } S_fS_F \;\&\; \text{S. ster. } S_nS_F$$

$$\text{S. ster. } S_fS_F \times \text{S. ster. } S_nS_F \longrightarrow \text{S. fert. } S_fS_n \;\&\; \text{S. ster. } S_FS_n$$

Modifying Factors

In so far as numbers and types of classes are concerned, the observed results are in strict accord with theoretical expectations. However, when we consider the ratios in which these different types appear, there is a wider departure from expectations. Nor is this at all surprising. Whether a plant shall be self-fertile or self-sterile is determined by the rate of pollen-tube growth in its style, and this is an exceedingly delicate reaction. Environmental

conditions affect it; East ('19) and Brieger ('27) have isolated genetic modifiers which change self-steriles into self-fertiles. If anything is at all surprising about the matter, it is the fact that, in a cross between parents differing in so many factors, and in dealing with a character so delicately adjusted as self-sterility, we should be able to find any genetic factors so clear-cut in their effect that their inheritance can be traced and their behavior in future generations confidently predicted.

As far as genetic modifiers are concerned, we should expect to get the greatest complexities in the second generation, for there recessive modifiers from either parent species would have a chance to recombine and turn otherwise self-sterile plants into self-fertiles, or vice versa. Next most difficult would be the back-crosses, while in the first generation we would expect the fewest complications. The results as reported below accord with these expectations. The only serious deviations from expectations are in the case of the second generation and in the back-cross to *N. Langsdorffii*.

In addition to the families grown in 1930 and 1931, there are the older records from 1924–26. These will all be considered together. In all the work pollinations were made in quadruplicate and when conflicting results were obtained the pollinations were repeated. The data will be discussed in the following order:

First generation—*N. Langsdorffii* × *N. alata*
Back-crosses to *N. alata*
N. alata × self-fertile F_1's
N. alata × self-sterile F_1's

Second generation
Self-fertile F_1 × self-fertile F_1
Self-fertile F_1 × self-sterile F_1
Self-sterile F_1 × self-fertile F_1
Back-crosses to *N. Langsdorffii*

FIRST GENERATION—N. LANGSDORFFII × N. ALATA

Data are at hand only for the original exceptional F_1, the result of pollinating *N. Langsdorffii* with the pollen of No. 15-2. We should expect self-fertiles (S_fS_n) and self-steriles (S_fS_f) in equal

numbers. The actual figures were twelve self-fertiles, sixteen self-steriles. The similar cross *Nicotiana* var. "Bowles" × *N. alata* 15-2 also yielded self-fertiles and self-steriles in approximately equal numbers.

BACK-CROSSES TO N. ALATA

N. alata × *self-fertile F_1's.*—Two families were grown in successive years, the results of pollinating two *N. alata* siblings by two different self-fertile F_1's. We should expect self-fertiles and self-steriles in equal numbers from such a mating. The actual results were as follows:

	$(S_xS_y) \times (S_fS_n)$	Number of self-fertile plants S_fS_x & S_fS_y	Number of self-sterile plants S_nS_y & S_nS_y
Family 8—1925	*N. alata* No. 35-1 × 29-7	26	17
Family 8—1926	*N. alata* No. 35-7 × 29-22	25	24

Cross- and self-fertility relationships could not be classified completely for Family 9-26, a cross between self-fertile F_1 No. 29-22 and *N. alata* No. 35-4, because it was segregating for an extreme form of male sterility in which no pollen was formed by some of the plants.

N. alata × *self-sterile F_1's.*—Four such families were grown. In each case we should expect self-fertiles and self-steriles in equal numbers. The results are as follows:

		Number of self-fertile plants	Number of self-sterile plants
Family 16—1925	29-6 × *N. alata* No. 35-2	19	22
Family 16—1926	29-8 × *N. alata* No. 35-2	24	20
Family 17—1925	*N. alata* No. 35-1 × 29-6	20	20
Family 17—1926	*N. alata* No. 35-1 × 29-8	19	17

SECOND GENERATION

Self-fertile F_1 × self-fertile F_1.—According to our interpretation, the genetic formula for the self-fertiles of the first generation was S_fS_n. On self-fertilization, or crossed with another self-fertile F_1, they should therefore have given all self-fertiles.

$$S_fS_n \times S_fS_n \longrightarrow S_fS_f + S_fS_n$$

This is on the hypothesis that the presence of the factor S_n in the tissues of the style will retard the growth of S_n pollen quite as effectively in a self-fertile plant as it would in a self-sterile plant. In case it did not do so a certain percentage of S_nS_n zygotes would result. As a matter of fact, two self-steriles did appear among some 200 seedlings, but in the absence of any precise data we can do no more than suggest that they may have arisen in this manner.

Self-fertile F_1 × self-sterile F_1 and *Self-sterile F_1 × self-fertile F_1*. —In each of these cases we should expect (in the absence of modifying factors) self-steriles and self-fertiles in equal numbers. In the first we should expect the following:

$$S_fS_n \times S_FS_f \longrightarrow \begin{matrix} S_fS_F & & S_fS_f \\ \& & + & \& \\ S_FS_n & & S_fS_n \end{matrix}$$

In the reciprocal cross, S_F would inhibit the pollen tubes carrying S_f with the following result:

$$S_FS_f \times S_fS_n \longrightarrow S_FS_n + S_fS_n$$

Five families were grown. The first two are from a self-fertile F_1 × a self-sterile F_1, the last three from the reciprocal combination.

		Number of self-fertile plants	Number of self-sterile plants
14—1925	29-7 × 29-12	29	9
14—1926	29-7 × 29-12	18	2
15—1925	29-12 × 29-7	27	13
15—1926	29-8 × 29-7	17	3
5N—1930	29-12 × 29-17	33	14
		124	41
Expectations on the hypothesis outlined below		124	41

In each case there is a serious deficiency of self-steriles. Since the general situation seems to be the same in all five families, we may treat with the total of 124 self-fertiles and 41 self-steriles. If there had been no complications we should have obtained 82 of each. Two recessive modifiers, however, such as those already described by East ('19), would have so changed part of the 82 self-steriles that in the absence of precise pollination tests with

plants of known constitution they would have been classified among the self-fertiles. On this interpretation, if we let r and r_1 represent the two recessive modifiers, *N. Langsdorffii* would have been of the constitution $S_fS_frrr_1r_1$ and *N. alata* 15-2 of the constitution $S_FS_nRRR_1R_1$. The first generation self-fertiles would therefore have been of the constitution $S_fS_nRrR_1r_1$, and their self-sterile siblings of the constitution $S_FS_nRrR_1r_1$. The cross between self-steriles and self-fertiles would be diagrammed as follows:

Self-sterile F_1 × Self-fertile F_1	Self-fertile combinations	Self-sterile combinations
S_FS_f Rr R_1r_1 × S_fS_n Rr R_1r_1	S_fS_n RR R_1R_1 — 1 S_fS_n Rr R_1R_1 — 2 S_fS_n RRR_1r_1 — 2 S_fS_n RrR_1r_1 — 4 $S_fS_n$rrR_1R_1 — 1 $S_fS_n$rrR_1r_1 — 2 S_fS_nRRr_1r_1 — 1 S_fS_nRrr_1r_1 — 2 $S_fS_n$rrr_1r_1 — 1	S_FS_f RR R_1R_1 — 1 S_FS_f Rr R_1R_1 — 2 S_FS_f RR R_1r_1 — 2 S_FS_f Rr R_1r_1 — 4 Total self-steriles 9
Modified self-steriles	$S_FS_f$rrR_1R_1 — 1 $S_FS_f$rrR_1r_1 — 2 S_FS_fRRr_1r_1 — 1 S_FS_fRrr_1r_1 — 2 $S_FS_f$rrr_1r_1 — 1	
Total self-fertiles and pseudo self-fertiles..... 23		

We should therefore expect a ratio of 23 self-fertiles to 9 self-steriles. That is, out of every 32 plants we would expect one-half to be true self-fertiles. The other half would be divided into 9 self-steriles and 7 modified self-steriles which would be fertile with their own pollen, but cross-sterile in certain combinations. For 165 plants the expectations of a 23:9 ratio are (in whole numbers) 124 to 41, which is the exact number actually obtained.

BACK-CROSSES TO N. LANGSDORFFII

This hypothesis can be tested by examining the back-crosses. Clearly, such modifiers could not have come from *N. alata*, since the ratios obtained in back-crosses to that species were quite as regular as in the first generation. Therefore to test our hypothesis we turn to back-crosses between an F_1 self-sterile and *N. Langs-*

dorffii. In the absence of modifying factors we should again obtain a 1:1 ratio between self-fertiles and self-steriles.

$$S_fS_f \times S_FS_f \longrightarrow S_FS_f + S_fS_f$$

If two recessive modifiers are present, we would obtain a ratio of seven self-fertiles to one self-sterile. The data are available from one such family and can be summarized as follows:

	Number of self-fertile plants	Number of self-sterile plants
Family 18—1926 26-3 × *N. Langsdorffii* No. 29-8	30	3
Expectations on hypothesis outlined above	(29)	(4)

While the data are too meagre for final conclusions to be drawn the results from the one back-cross family are consistent with the results from the second generation families. Both point to *N. Langsdorffii* as having introduced recessive modifiers into the cross, which upon recombination in the second generation and in the back-cross to *N. Langsdorffii*, turned nominal self-sterile individuals into apparent self-fertiles. The ratios from both types of families are consistent with the hypothesis that two such recessive modifiers were introduced from *N. Langsdorffii*.

LINKAGE BETWEEN THE SELF-STERILITY ALLELOMORPHS AND OTHER GENES

Family No. 5N-30 was a second generation from a cross between *N. Langsdorffii* and *N. alata*. These two species (or sub-species) differ by a large number of other characters beside self-sterility and self-fertility. Figure 5 and pl. 4 show typical flowers of each species. Some of the most outstanding differences are set out below in tabular form:

N. Langsdorffii	*N. alata*
Flowers green	Flowers white
Corolla-tube short	Corolla-tube long
Style proportionately short	Style proportionately long
Pollen blue	Pollen ivory

Fig. 5. Typical flower of *N. alata* (left) and of *N. Langsdorffii* (right) drawn to the same scale.

Nor are there any evident cytological complications which would hinder free recombination. Examination of the pollen mother cells with iron-aceto-carmine showed the reduction divisions to be regular. Certainly there was not more irregularity than existed in either of the parent species. Since the parent species differed in such a large number of characters it was thought probable that some of them would show linkage with the self-sterility allelomorphs. Such linkage has been reported for *Nicotiana* by Brieger and Mangelsdorf ('27) (anthocyanin flower color) and for *Antirrhinum* by Brieger ('30). Each plant of Family 5N-30 was accordingly scored for flower color, pollen color, tube length, and style length.

Corolla color.—As has been determined by a number of investigators the difference between the green-plastid corolla of *N. Langsdorffii* and the pure white-plastid corolla of *N. alata* is mainly due to a single factor, green being a simple dominant to white. The data

TABLE IV

NUMBER OF PLANTS OBTAINED IN A SECOND-GENERATION CROSS OF GREEN SELF-FERTILE AND WHITE SELF-STERILE

	Number of self-fertile plants	Number of self-sterile plants	Total
Green plastids	26 (25.3)*	10 (10.7)	36
White plastids	7 (7.7)	4 (3.3)	11
Total	33	14	47

* The figures outside the parentheses indicate the actual number of plants obtained. The figures within the parentheses show the number to be expected if self-sterility is inherited independently from plastid color.

from 5N-30 were in accord with this interpretation and also indicated that there is no appreciable linkage between the factor for plastid color and the self-sterility allelomorphs, S_1, S_2, S_f, etc. The data are given in table IV.

Corolla-tube length.—As is shown in fig. 5 and in pl. 4, this is the most conspicuous difference between the two parental species. It is due to at least four or five main pairs of factors and a number of minor modifying factors. Since there are only nine pairs of chromosomes in *N. alata* and *N. Langsdorffii*, the chances are good that at least one pair of factors affecting tube length might be linked with the self-sterility allelomorphs. From purely *a priori* assumptions we should therefore expect, upon crossing the long-tubed self-sterile species with the short-tubed self-fertile one, to find a higher percentage of self-steriles among the longest-tubed members of the second generation than among the shorter-tubed ones. The actual figures are as follows:

Tube length in millimeters	Number of self-fertile plants	Number of self-sterile plants	Per cent of self-sterile plants
30–39	12	3	20
40–49	16	6	27
50–59	5	5	50

Style length.—In *N. Langsdorffii* the style is shorter than the tube; in *N. alata* it is often much longer and the protruding stigmas are very conspicuous. While this is a highly variable character, even on a single plant, it can be roughly classified for purposes of comparison. The plants of 5N-30 were recorded as short-styled (like *N. Langsdorffii*) or long-styled (like *N. alata*) or intermediate. From the behavior of the character in later generations it is clearly affected by several pairs of factors. It is not surprising therefore that a higher percentage of the longer-styled plants were self-sterile, as the following table shows:

Proportional length of style	Number of self-fertile plants	Number of self-sterile plants	Per cent of self-sterile plants
Short	11	2	15
Intermediate	4	1	20
Long	18	11	38

Pollen color.—The pollen of *N. Langsdorffii* is a bright dark blue, that of *N. alata* is ivory or cream-colored, though the stamens themselves are often dark. The pollen of the F_1 plants was intermediate in color. In the second generation dark blue, ivory, and intermediates resembling the F_1 could be distinguished. The segregation is fairly clear-cut; probably not more than two or three pairs of factors are involved. One of them is evidently quite strongly linked with the sterility allelomorphs. It will be seen that we did not obtain a single self-sterile plant with dark blue pollen.

Pollen color	Number of self-fertile plants	Number of self-sterile plants	Per cent of self-sterile plants
Blue	7	0	0
Intermediate	19	7	27
Ivory	7	7	50

From the above discussion it is clear that in *Nicotiana* a number of genes are linked closely enough with the self-sterility allelomorphs to be detected readily. Linkage has been demonstrated and the linkage intensity calculated for the gene for anthocyanin flower color by Brieger and Mangelsdorf ('26). In the data reported above the linkage of one of the genes controlling pollen color and of at least one each of the genes for tube length and for proportional length of style is indicated. A careful study of other multiple factor differences between *N. Langsdorffii* and *N. alata* (as, for instance, leaf shape and stipule decurrence) would greatly extend the list.

For all of these genes Family 5N-30, a cross between two first-generation plants, was more like an ordinary back-cross than a normal second generation, due to the influence of the self-sterility allelomorphs. This fact is brought out diagrammatically in fig. 6, where the relationships of genes and the proportional contributions of the two parental species to the second generation are diagrammed, first, for an ordinary pair of Mendelian factors, and second, for the self-sterility allelomorphs S_F, S_f, etc. In the case of ordinary genes, if we consider the second generation as a whole, the two parental species have made equal contributions. In the case of the self-sterility allelomorphs all the male gametes carrying the S_f factors from *N. Langsdorffii* have been stopped.

As a result three-quarters of the self-sterility genes of the second generation, considered as a whole, have had their origin from *N. alata* and only one quarter from *N. Langsdorffii*. As far as the self-sterility allelomorphs are concerned the second generation Family 5N-30 was a back-cross to *N. alata*. To a lesser extent this was true as well for all the genes linked with the self-sterility allelomorphs.

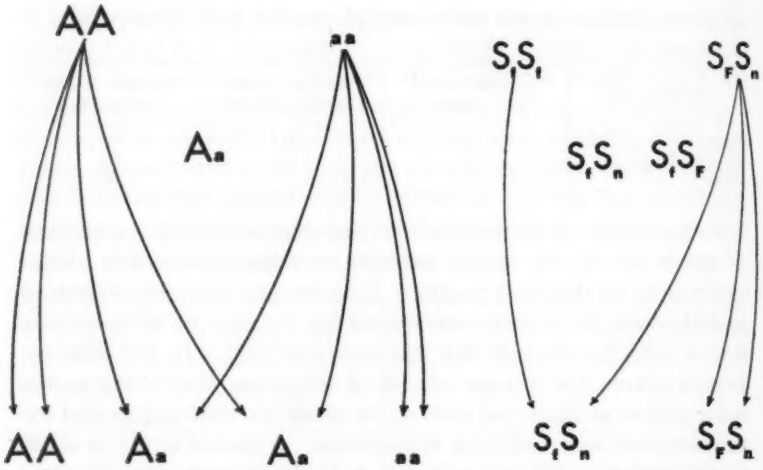

Fig. 6. Proportional contributions to the F_2: in the case of an ordinary pair of Mendelian factors (left); and in the case of the self-sterility allelomorphs (right).

The net result of such linkage should be quite striking in a species cross. The magnitude of the effect would depend upon the ratio between the non-crossed-over chromosome segment carrying the S factor and the chromatin as a whole. If we let n represent the average number of such segments then n-1 will represent that part of the chromatin not linked with the S factors. In the second generation this much of the chromatin will segregate normally and the relative contributions of the two species will be equal. If we let L stand for *Langsdorffii* chromatin and A for *alata* chromatin, the composition of the second generation can be represented as 2(n-1)L + 2(n-1)A. For the segment containing the S factors, segregation will be abnormal. If the cross was made

as in the case of Family 5N-30, the composition of the second generation for this segment of chromatin will be 1L + 3A. Combining the two totals, the proportion of L and A chromatin in the second generation will be 2(n-1)L + 1L: 2(n-1)A + 3A which simplifies to (2n-1)L : (2n+1)A. In the absence of any genetical or cytological data as to the frequency of crossing-over in *Nicotiana* we can go no further with certainty. We know, however, that there are nine pairs of chromosomes. If, just as a guess, we take the average cross-over length as one-third of a chromosome, n will equal twenty-seven and the proportion of L and A chromatin in Family 5N-30 will be 53L : 55A.

That this is no mere idle speculation is proved by a comparison of East's ('16) tables and plates with those presented in this paper. In each case a second generation between *N. alata* and *N. Langsdorffii* was studied. In his case the selective effect of the S factors, if any, was in favor of *N. Langsdorffii*. In the cross reported here it was in favor of *N. alata*. And the tables and plates show that 5N-30 was definitely more like *N. alata* than the second generation families figured by him. Precise comparison is possible only for the length of corolla-tube. The data from both crosses are assembled graphically in fig. 7. Family 5N-30 is definitely more like *N. alata* than was East's F_2. The difference in the shape of the two curves is quite as striking as their position and is equally significant.

It may be remarked in closing that the complications introduced by self-sterility factors will be more striking in the case of species crosses than in crosses between closely related strains. In the latter case such anomalies as are due to linkage with the self-sterility allelomorphs will be apparent mainly in the ratios obtained between different types of offspring. In the case of species crosses, however, the chromatin in the neighborhood of the self-sterility allelomorphs will have accumulated a whole set of differing genes in the two species. In hybrids between them, reciprocal crosses may be characterized by gross morphological differences. This possibility has been alluded to by Brieger in his recent monograph ('30) and is, according to his brief reference, the explanation of the differences obtained in reciprocal crosses in *Antirrhinum* by Lotsy ('12) and Baur ('11).

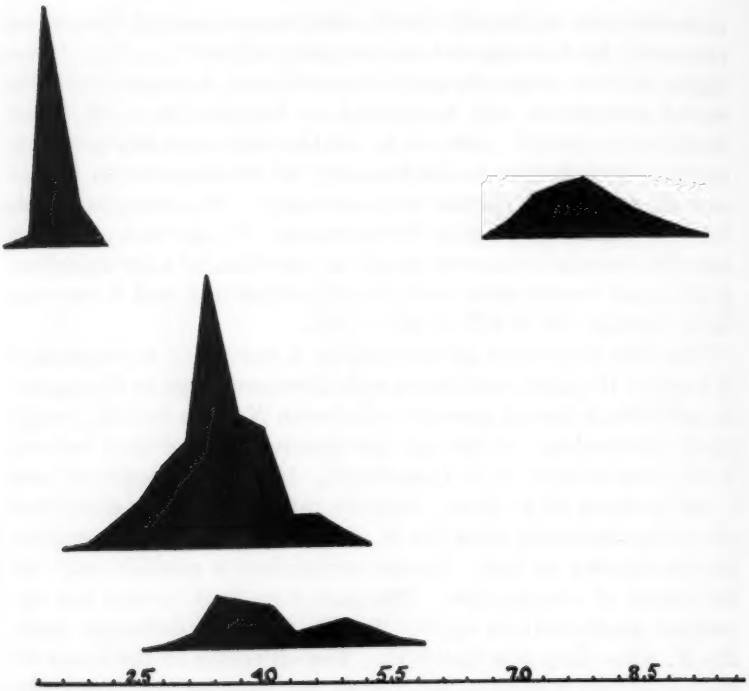

Fig. 7. Corolla length in centimeters for:

N. Langsdorffii	(upper left)	56 individuals	(data from East).
N. alata	(upper right)	49 individuals	(data from East).
East's F_1	(center)	163 individuals	(data from East).
Family 5N–30	(lower center)	47 individuals.	

(Each division of the scale represents 3 mm.)

SUMMARY

I. In a number of crosses between self-fertile and self-sterile species of *Nicotiana*, a single plant of *Nicotiana alata* gave the following anomalous results:

1. It was female sterile when pollinated with self-fertile Nicotianas.

2. When used as a pollen parent with self-fertile Nicotianas half of the first generation hybrids were self-sterile.

3. These exceptional F_1 self-steriles were (as females) cross-sterile with their self-*fertile* parents!

II. These anomalies are interpreted as due to a single factor, S_F, belonging to the allelomorphic series S_f, S_1, S_2, S_3, S_4, etc., studied by East and his students. S_F has the same properties as the self-sterility allelomorphs previously described; that is, its presence in the cells of the style inhibits the growth of pollen tubes carrying the same factor. It differs from the factors hitherto described in that it also inhibits all pollen carrying the full fertility allelomorph S_f.

III. The F_1, F_2, and back-cross ratios of self-fertile to self-sterile are consistent with the assumption that two recessive modifying factors were introduced into the cross from *N. Langsdorffii*. When homozygous they turn otherwise self-sterile plants into apparently self-fertile plants (pseudo-self-fertiles).

IV. The S allelomorphs were found to be independent of the factor for green corolla color (plastid color). They are linked with one of the factors for pollen color and with at least one of the factors for length of corolla tube and for proportional length of style.

Attention is called to certain complications introduced into inter-species crosses by the interaction of the self-sterility allelomorphs. The morphological differences between the *Langsdorffii-alata* F_2 studied by East and the second generation family of the present experiment are interpreted on this basis.

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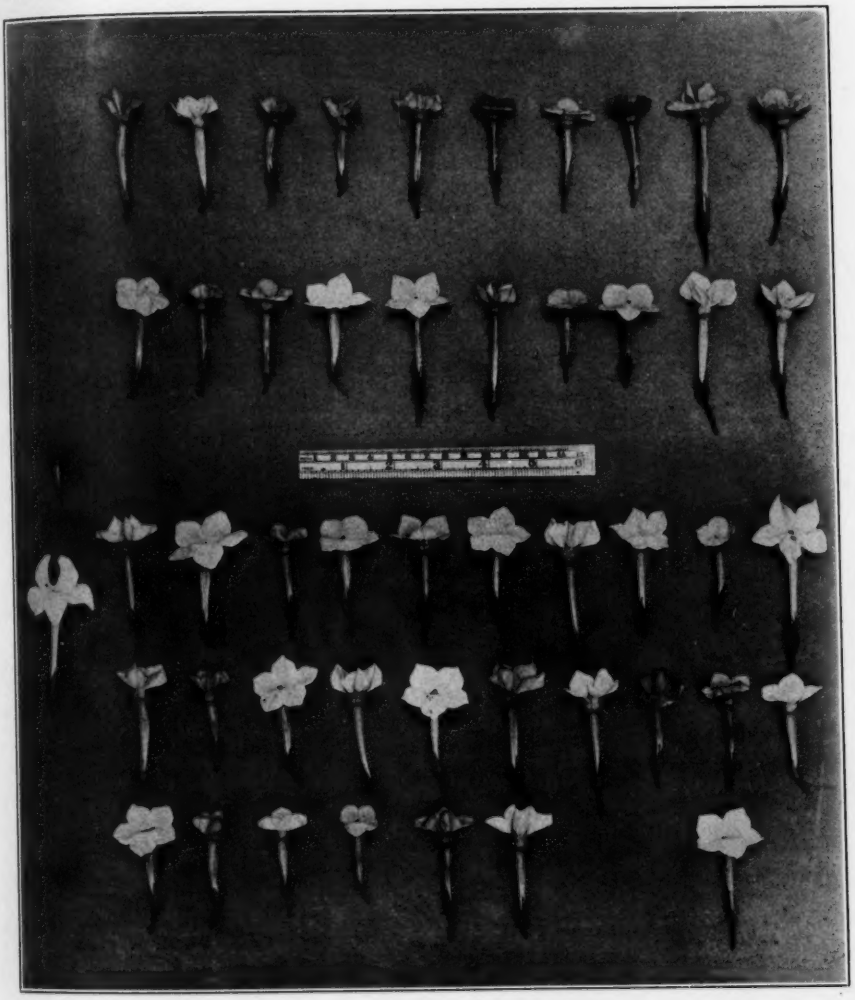
Explanation of Plate

PLATE 4

One flower each from the plants of Family 5N–30, a second generation from the cross *N. Langsdorffii* × *N. alata*. At the extreme left single flowers of the two parent species for comparison.



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